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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 13/00

A1

(11) International Publication Number: **WO 96/39493**

(43) International Publication Date: 12 December 1996 (12.12.96)

(21) International Application Number: PCT/US95/12343

(22) International Filing Date: 27 September 1995 (27.09.95)

(30) Priority Data:
08/466,437 6 June 1995 (06.06.95) US

(60) Parent Application or Grant
(63) Related by Continuation
US Not furnished (CIP)
Filed on Not furnished

(71) Applicant *(for all designated States except US)*: U.S. ENVIRONMENTAL PROTECTION AGENCY [US/US]; 401 M Street, S.W., Washington, DC 20460 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BLACKMAN, Carl, F. [US/US]; 3413 Horton Street, Raliegh, NC 27607-3414 (US). BLANCHARD, Janie, Page [US/US]; 1226 Trestle Glen Road, Oakland, CA 94610-2523 (US).

(74) Agent: KORNBAU, Anne, M.; Browdy and Neimark, Suite 300, 419 Seventh Street N.W., Washington, DC 20004 (US).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, MW, SD, SZ, UG), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: METHOD AND APPARATUS FOR ALTERING IONIC INTERACTIONS WITH CHEMICALS AND CHEMICAL PROCESSES USING MAGNETIC FIELDS

(57) Abstract

Chemical and biological systems can be altered, enhanced or suppressed, in a precisely controllable manner by exposure to a predetermined combination of AC and DC magnetic fields. The flux density of the DC magnetic field can be minimized perpendicular to the AC magnetic field while the DC magnetic field flux density parallel to the AC magnetic field is adjusted to the value required to make an ion or ions of interest resonate. The flux density of the AC magnetic field can be ajdusted to produce a controlled degree of response in a chemical or biological system.

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METHOD AND APPARATUS FOR ALTERING IONIC INTERACTIONS WITH CHEMICALS AND CHEMICAL PROCESSES USING MAGNETIC FIELDS

Field of the Invention

The present invention is directed to a method for using magnetic fields to alter ionic interactions with chemicals and chemical processes.

The present application is a continuation in part of serial no. 08/329,980, filed October 27, 1994, the entire contents of which are hereby incorporated by reference.

Background of the Invention

The results of a number of studies suggest that low-intensity and low-frequency electric and magnetic fields may influence physiologic processes in biological systems. However, most theoretical models developed to date have been unable to establish a predictive association between low-intensity field exposure and biological results. Some models of electric and magnetic field interactions with biological systems, for example, have focused on endpoints associated with direct energy deposition into the system from the fields or from the induction of body currents, and suggest that a single variable, such as AC field intensity, is responsible for the observed results. Partially as a result of these incomplete models, many experimental reports fail to document all relevant field exposure parameters and do not establish a clear protocol for obtaining repeatable results. Inconsistencies between experimental results have subsequently been interpreted by some as evidence that electric or magnetic fields may not be the causal factors (e.g., Adair, 1991; 1992). While there is much theoretical support for resolving AC and DC fields into parallel and perpendicular components in order to determine how they will affect biological systems, experimental efforts often fail to document the relative orientation between the AC and DC fields. In other experiments, different field variables such as frequency, temporal duration of fields, and relative alignment with the local geomagnetic field have been characterized on an ad hoc basis without clear guidance from a theoretical model to indicate which parameters were critical (Adey, 1992; Blackman et al., 1985, 1988, 1990; Blackman, 1992;

Liboff, 1985, 1992; Liboff et al., 1987; Smith et al., 1987; Thomas et al., 1986).

A variety of theoretical models have been developed to describe the interaction of different combinations of static (DC) and extremely-low-frequency time-varying (AC) magnetic fields with living systems. In fact, most theoretical works, including quantum mechanics texts (e.g. Yariv, 1982), focus exclusively on how an AC magnetic field oriented perpendicular to the DC magnetic field will alter the spin of an ion. Edmonds (1993), for example, recently developed a model that concentrated on the case of perpendicular AC and DC fields. Most of the above-described models are largely descriptive, without being predictive.

The ion cyclotron resonance (ICR) model, originally formulated by Liboff (cf. Liboff, 1985, McLeod and Liboff, 1987) and discussed by Durney (1988), Halle (1988) and Sandweiss (1990), describes how unhydrated ions might have distinct resonance type responses caused by the local DC magnetic field.

The fundamental premise of the ICR model is that parallel magnetic fields tuned for calcium, or a limited set of other selected ions, enhance the passage of those ions across the plasma membrane of the cell, only when $B_{ac} = B_{dc}$.

Theoretical support for the plausibility of measurable biological effects occurring as a result of exposure to parallel DC and AC magnetic fields can be found in the work of Chiabrera and colleagues (Chiabrera and Bianco, 1991; Chiabrera et al., 1991, 1993; Bianco and Chiabrera, 1992). They applied their model to a variety of biologically active ions in addition to calcium using the charge to mass ratio for the unhydrated state, a condition that may exist in ion-ligand components of biological molecules. Chiabrera and colleagues suggested that ions affected by ICR model conditions might be located in binding sites formed by molecular crevices that would exclude hydration of the ions. Although the ICR model predicts enhanced responses by specific ions when the AC frequency corresponds with the ICR model conditions, which are different for each ion, it does not indicate how the response

might vary with different AC flux densities. Thus, the ICR model does not anticipate the distinct response form subsequently predicted for increasing B_{ac} at constant B_{dc} and f_{ac}.

Lednev (1991) incorporated Liboff's model, in a limited sense, in his examination of how parallel AC and DC magnetic fields might influence ions bound in ligand structures specific to Ca^{++}.

The ion parametric resonance (IPR) model differs from Lednev's model in three critical ways: it specifically includes a $(-1)^n$ term multiplying the Bessel function prediction, the IPR model Bessel function argument is twice that of the Lednev model, and the IPR model considers a wider range of candidate ions, through an expanded understanding of the role of the ion in creating a biologically significant change.

The IPR model considers the potential effects on any unhydrated ion, presumably bound within a molecular structure, that can influence the observed biological response. The molecular structure may be composed of proteins, nucleic acids, or lipids, either singly or in any combination, as long as the structure itself requires an ionic cofactor to function. Extension to unhydrated ions beyond Ca^{++} can be inferred in part by the work of Liboff (1985, 1992) and Chiabrera and colleagues, op. cit.

Background Art

Tissue and cell development have been studied extensively to determine the mechanisms by which maturation, maintenance and repair occur in living organisms. Generally, development of a cell or tissue can be considered as a transformation from one state or stage to another relatively permanent state or condition. Development encompasses a wide variety of patterns, all of which are characterized by progressive and systematic transformation of the cells or tissue.

In many instances it is desirable to control or alter the development of cells and tissue *in vivo*. It is hoped that means can be provided to restore or maintain the natural

order of an organism after a debilitating injury, disease or other abnormality.

As will be appreciated by those skilled in the art, tissue and organic development involve complex processes of cellular growth, differentiation and interaction mediated by complex biochemical reactions. At the genetic level, development is regulated by genomic expression; at the cellular level, the role of membrane interaction with the complex biochemical milieux of higher organisms is instrumental in development processes. Moreover, remodeling of tissues or organs is often an essential step in the natural development of higher organisms.

A role for biologically active ions in cellular activity is well established. In Liboff et al., U.S. Patent No. 4,818,697, techniques are disclosed for controlling the movement of a preselected ionic species across the membrane of a living cell. The inventors disclose that by exposing a region of living tissue of a subject such as a human or animal to an oscillating magnetic field of predetermined flux density and frequency, the rate of tissue growth can be controlled. For stimulating bone growth rate, a fluctuating magnetic field is tuned to the specific cyclotron resonance frequency of a preselected ion such as Ca^{++} or Mg^{++}. Additionally, Liboff et al. in U.S. Patent No. 4,932,951, disclose the use of cyclotron resonance tuning to control the growth rate of non-osseous, non-cartiliginous connective solid tissue. In U.S. Patent No. 5,067,940, Liboff et al. disclose a method and apparatus based on cyclotron resonance tuning which allow the growth rate of cartilaginous tissue to be regulated. An even more important use of cyclotron resonance tuning which is of particular significance in the treatment of elderly patients is disclosed in Liboff et al. U.S. Patent No. 5,100,373, which deals with a method and apparatus for treating and preventing osteoporosis, both locally and systemically. Additional patents granted to Liboff and his co-workers in the field of ion cyclotron resonance include U.S. Patents 4,818,697; 4,932,951; 5,045,050; 5,059,298; 5,067,940; 5,077,943; 5,087,336; 5,088,976; 5,100,373; 5,106,361; 5,123,898; 5,143,588; 5,160,591, and

5,193,456. All of the above-cited patents are hereby incorporated by reference in their entirety. These patents address various applications of the concept of field induced changes in ion transport in biological systems. The primary requirement for these applications is for a time varying (AC, preferably sinusoidal) magnetic field and a static magnetic field oriented parallel to the AC field. Liboff postulates, without explicit theoretical support, that the maximum influence will occur when $B_{ac}=B_{dc}$. Furthermore, there is the requirement for specific frequencies of AC field to tune to resonance conditions for particular ions of interest.

Deficiencies in Background Art

Although for a long time it has been postulated that magnetic fields have potential effects on biological systems, there has been no clear evidence to date indicating the critical parameters influencing the effects. As a result, replication of observed effects has been limited at best. Lacking clear indication of the possible causes and forms of magnetic field influence, a linearly increasing effect with increasing AC field strength was assumed. However, the present inventors have discovered that under the specific conditions identified herein, neither this assumption nor the postulated maximum effect when $B_{ac}=B_{dc}$ is correct.

The work by Liboff et al. (1987) describing the transport of calcium/magnesium ions across a membrane of cells and bones considers specifically the physical motion of those ions as a result of the application of magnetic fields whose effect is to transform the random motion of those ions to a path matching the geometric form of the spiral channel postulated to provide passage across the membrane. Although the "characteristic resonance frequency" of the IPR model is identical in mathematical form to that of the ICR model resonance, it will be seen that resonance as defined for the IPR model of this application is the mathematical inverse of that defined for the ICR model. Further, the mechanism of interaction postulated for the IPR model is distinct from that of the ICR model by virtue of abandoning a geometric concept and focussing on the ion's role within a molecular structure,

such as an enzyme, protein, nucleic acid, and that the IPR model's consideration of candidate ions is therefore broader than is that of the ICR model.

Sandyk (1993) examined the application of magnetic fields to influence the pineal gland in patients with Parkinson's disease to moderate the melatonin caused hyperglycemia. However, the AC field flux densities applied were substantially below those postulated to be effective by the IPR model, assuming an approximate geomagnetic source of the ambient DC magnetic field. Further, three different orientations for the applied AC fields, with respect to a presumably fixed DC field, were required to produce an effect, obviating any requirement for parallel fields.

The work of Liburdy et al. (1993) also demonstrated the use of AC magnetic fields to control the influence of melatonin without a clear indication of the AC magnetic field orientation with respect to that of the ambient DC magnetic field. Lerchl et al. (1991) specifically considered the influence of parallel fields on pineal gland function, showing that a single selected combination of fields reduced the synthesis and production of melatonin. This single data point is between the maximal effect and null effect predictions by the IPR model, assuming Ca^{++} resonance. Lerchl further considers the distinction between parallel and perpendicular fields, postulating them to follow a cosine law form. Although these works, taken together, appear to suggest a likely effect on either melatonin production, or its action in vivo, or both, they each give results for single combinations of applied magnetic fields without any further guidance for how these effects might change with variations in AC flux density, frequency, or DC flux density. As will be shown below, no person of ordinary skill in the art at the time of these publications would have been motivated to demonstrate the parallel AC and DC magnetic fields would be able to control the function of melatonin, either applied or as produced by the pineal gland, in the controlled and distinctly predicted non-linear form predicted by the IPR model. Further, no person of ordinary skill in the art at the time of these publications

would have been motivated to demonstrate the three distinct responses shown under parallel (on or off resonance) and perpendicular AC and DC magnetic fields, indicated by the present application, across a critical range of AC flux densities.

Absent from any of the aforementioned arts is any recognition of:

(a) the critical influence of the strength (flux density) of the AC magnetic field on the magnitude of magnetic field influence on a biological system;

(b) the importance of reducing the static magnetic field perpendicular to the AC magnetic field to near zero value in order to distinctly get the IPR model predicted result;

(c) the potential for a single exposure condition to differentially stimulate multiple ions concurrently;

(d) evidence of the unique role of a variety of otherwise biologically significant ions, including but not limited to hydrogen, sometimes critical, in a biological system's response to a magnetic field;

(e) the explicit recognition of peak AC field measurements (in contrast to rms) as the appropriate metric.

(f) a clearly prescriptive identification of how the system might differentially respond to variations in B_{ac} (with B_{dc} and f_{ac} constant) except for a postulated effect when $B_{ac} = B_{dc}$ (with B_{ac} interpreted by some experimenters as rms and by others as peak).

(g) indication of the distinction in biological/chemical system response between exposure to parallel and exposure to perpendicular AC and DC magnetic fields, and the critical importance of maintaining strictly parallel fields in order to get the distinct response form predicted by IPR model.

It has also been found that the ion cyclotron resonance condition disclosed by Liboff is a special case of the ion parametric resonance model that is not extendable via harmonics, as Liboff and others have assumed to date.

In both the Lednev and the IPR model of application Serial No. 08/329,980, magnetic field interactions with ions

are characterized by a frequency index, n, defined as the ratio of the (ion specific) cyclotron resonance frequency to the applied AC frequency. When n for a given ion has integer value, the system is said to be in resonance for that ion. When n = 1, resonance is also found with the ICR model. However, because resonance is determined by an ion's charge to mass ratio (q/m), none of these models can distinguish effects from ions with similar q/m values, or from ions with q/m values that are integer multiples of the q/m value for other ions. Further, if different ions are at resonance under a given magnetic field exposure, each could be supporting, opposing, or have no effect on the selected observable ion.

Summary of the Invention

It is an object of the present invention to overcome the aforesaid deficiencies of the prior art.

It is another object of the present invention to define all of the magnetic field exposure conditions that are required to produce chemical and biological effects.

It is another object of the present invention to change a selected response of a chemical or biological system (enzyme, cell, organ, or organism) containing at least one ion that influences that selected response by creating a resonance for one or more of those ions having biological significance, according to the precise definition for resonance given in the text below.

It is a further object of the present invention to provide an influence on a selected biological/chemical system which oscillates between maximal and no effect in a mathematically well-defined non-linear manner as a function of the AC magnetic flux density only when the system can be said to be at resonance for a biologically significant (to the particular system) ion.

It is a further object of the present invention to provide a means for precisely controlling the degree of response of a biological/chemical system to an externally imposed condition (i.e. defined combinations of AC and DC magnetic fields, applied chemicals, or both).

It is a further object of the present invention to provide a means of changing the effects described above for specific combinations of AC and DC magnetic fields by reorienting the AC and DC magnetic fields with respect to each other.

It is a further object of the present invention to create three biological/chemical response options in a treated system for selected AC and DC magnetic field flux densities and AC frequency: (1) on-resonance parallel AC and DC magnetic fields (creating, for example, maximal inhibition of a biological response), (2) off-resonance parallel AC and DC magnetic fields (creating no change in the biological/chemical system in comparison to the unexposed case), and (3) on-resonance perpendicular AC and DC magnetic fields (creating, for example, maximal increases in the biological response).

The present invention provides a method for altering ionic interactions in systems including cells and organisms using magnetic fields. The method involves controlling the orientation and varying the intensity, and fluctuation frequency of paired static and sinusoidally varying magnetic fields in such a way as to create certain magnetic interactions between ions and the molecules with which they are associated. The magnetic fields can be adjusted to control precisely the desired orientation, intensity and fluctuation frequency of the magnetic fields.

The desired parameters are calculated from a mathematical model developed to quantify the interactions of magnetic fields with ions and their associated molecules. The method which comprises the present invention is based upon application of this mathematical model. By using this mathematical model, the present invention makes it possible to determine parameters necessary for causing particular ion-molecule interactions and can accurately define the desired magnetic fields. The invention thus can be used for non-destructive characterization and evaluation of chemical and biological systems.

The ion parametric resonance (IPR) mathematical model of the present invention examines biological responses to

parallel AC and DC magnetic fields and, by specifying the functional influences of all magnetic field parameters, provides detailed predictions of the expected atomic level responses.

According to the present invention, the influence of the strength (flux density) of an AC magnetic field oscillates between maximal and no effect as the AC field is increased, not in direct proportion to the magnitude of the AC field, but as a function of a Bessel function of the first kind whose argument involves the ratio of the frequency index (defined within) and the magnitudes of the AC and the DC magnetic fields. The static magnetic field that is perpendicular to the AC magnetic field must be reduced to near zero to avoid alternative interaction with the phenomena. The static magnetic field is used for the identification of multiple ions for which this effect occurs, and the multiple chemical systems differentially stimulated concurrently using a single exposure condition, as well as a crucial role for hydrogen ions, which had heretofore been unrecognized.

According to the present invention, alternative forms of response by a chemical or biological system are possible by adjusting the relative amount of B_{dc} perpendicular to B_{ac}. The addition of a perpendicular B_{dc} can dramatically attenuate or even eliminate the response caused by parallel B_{ac} and B_{dc}. Substituting a B_{dc} perpendicular to the B_{ac} in place of the parallel B_{dc}, called for in the IPR model situation, can cause a reversal in the direction of the response in a chemical or biological system.

The present invention is particularly useful in at least four fields:

(1) Diagnosis.

Methods for determining which ions, and their oxidation states, are involved in chemical and biochemical complexes and reactions. The effect may be observed by using a direct physical method for detection, e.g., molecular conformation or molecular dynamics techniques, or indirect methods that look at reaction products or biological responses.

(2) Altered Reactions and Processes.

Methods for altering ion associated chemical and biochemical complexes and reactions, including changes in the balance between alternative, competing chemical pathways that lead to a different mix of products. In addition, alterations may occur in any process that has an ion as part of a regulatory control mechanism, as in the case of ion gates in some protein channels in cell membranes.

(3) Changing Biological Processes.

Methods for altering biochemical processes which change an organism's response to environmental agents or influences. Any process that involves ion interactions with biological molecules is potentially subject to control or alteration, including such diverse actions from animal/human response to endogenous or exogenous chemicals, e.g., opioids, and to alterations in acquisition of learning and retention of memory.

(4) Delivery/Activation Processes.

Methods for triggering chemical or biochemical reactions in localized regions of space. For example, biologically active compounds attached to carrier molecules or encased in vesicles designed with ionic cofactors may be released at particular sites in the body when the kinetic interactions of the particular ions with the carrier or vesicle are altered by appropriate exposure to magnetic fields. The biologically active compounds may include an active component complexed with an inactivating agent, which includes an ion cofactor. The compounds are then rendered active or inactive, as desired, when the ionic interaction is changed by appropriate magnetic field exposure conditions with in a specific volume of space.

Brief Description of the Drawings

Figure 1 shows the results of Test 1 for inhibition of percent neurite outgrowth stimulated by nerve growth factor in PC-12 cells exposed to 45 Hz sinusoidal magnetic fields between 77 and 200 mG(rms){108-283 mG(pk)}.

Figure 2 shows the results of Test 2 for reduced effectiveness of 45Hz sinusoidal magnetic fields between 200

and 468 mG(rms){284-662 mG(pk)} to inhibit neurite outgrowth stimulated by nerve growth factor in PC-12 cells.

Figure 3 shows the results of Test 3 for the response of nerve growth factor-stimulated PC-12 cells to 45 Hz, sinusoidal magnetic fields under non-resonance conditions.

Figure 4 shows the results of Test 4. Figure 4a shows a comparison of neurite outgrowth in cells stimulated by nerve growth factor and exposed to sinusoidal magnetic fields under resonance conditions by AC exposure frequency (45 Hz vs. 25 Hz where the 25 Hz responses are connected by lines) but plotted without regard for the different B_{dc} flux densities that are present in the 45 Hz v. 25 Hz cases. Figure 4b shows the results from each run when the different B_{dc} flux densities are included as part of the independent variable (plotted on the horizontal axis) to indicate the IPR model predicted Bessel function argument.

Figure 5 shows the ad hoc fit of an IPR model to acquired data. Figure 5a shows the general fit of n=1 prediction of IPR model to data acquired from tests 1, 2 and 4. Figure 5b shows the near constant results across the range of B_{ac} for off-resonance conditions, Test 3.

Figure 6 shows a best fit to data using the simplest interpretation of the IPR model: a weighted sum of Bessel function responses selected by ions at or very near resonance.

Figure 7 shows an improved fit when the response associated with the hydrogen ion is considered exclusively for the low B_{ac} response, with a conversion to a weighted sum of Bessel functions at a mathematically determined optimal coversion point. This result suggested a special role for the hydrogen ion, that was later confirmed by other experimentalists (Trillo et al., 1994). In that work, the conversion point identified here also showed a critical distinction in the data.

Figure 8 demonstrates continued consistency of PC-12 cell responses with IPR model predictions under specific IPR model prescribed combinations of AC and DC magnetic fields. The IPR model fit to the data in Figure 7 was extended to higher values of B_{ac}, where the results of experimental tests

at those values were then plotted. This match of the data to such an unusual predicted response form is extremely unlikely to occur by chance.

Figure 9 shows that cells prepared in the standard medium, RPMI 1640, exhibited the IPR model anticipated response form whereas those prepared in Iscoves' medium exhibited a minimal response, nearly indistinguishable from an IPR model predicted off-resonance response form, although the magnetic field exposure conditions for each of these test were essentially identical. This result is consistent with the effect of magnetic fields being localized to the cell plasma membrane.

Figure 10 demonstrates an alternate biological/chemical system response to IPR model specified conditions. Rat liver cells in culture (clone 9) exhibit gap junctional intercellular communication by transferring a fluorescent dye away from the site of application, which is quantified by scoring the number of rows away from the application point that are stained with the dye. Under the influence of IPR exposure conditions similar to those described for neurite outgrowth in PC-12 cells, the expected U-shaped exposure response is observed. Although less detailed than the PC-12 measurements, these data show, in a preliminary way, the reasonableness of extending the IPR model to systems other than PC-12 cells.

Figure 11 shows the neurite outgrowth response of cells exposed over a field-strength range of 45-Hz AC magnetic fields under various different B_{dc} orientations and flux densities. The mean and 2 · SE of the percent neurite outgrowth (%NO) is plotted for four different DC magnetic field conditions. H(5 trials) is for 366mG perpendicular, <2mG parallel DC magnetic fields. H&V(3 trials) is for 366mG perpendicular, 366mG parallel DC magnetic fields. 0.4H&V(3 trials) is for 160mG perpendicular, 366mG parallel DC magnetic fields. V(3 trials) is for <2mG perpendicular, 366mG parallel DC magnetic fields. These results demonstrate that the orientation and flux density of the B_{dc} relative to the B_{ac} can negate parallel field resonance conditions, and even reverse

the cell response for strictly perpendicular B_{ac}/B_{dc} under specific exposure conditions.

Detailed Description of the Invention

It is well established that biological activity is driven by enzymatically controlled chemical reactions, and that some enzymes incorporate specific ions as cofactors to initiate or modulate their reaction rates. The role of other specific ions can be seen in selective functions of proteins, such as those involved in electron or oxygen transport.

Ionic cofactors and reaction centers and their dynamic interactions driven by thermal motion are critical elements in biological activities. At biologically relevant temperatures the enzyme molecules are immersed in a bath of solute molecules vibrating at infrared frequencies. Thus, it follows that ionic cofactors, their associated enzymatic reaction centers, and their dynamic interactions driven by the ever present thermal bath, are critical elements in biological activities. "Thermal" means the average translational kinetic energy of molecules. Native proteins at biologically relevant temperatures are not static forms, but fluctuate constantly, passing through a variety of similar configurations due to thermal influence. Karplus and Petsko (1990) point out the importance of this kinetic view of proteins by stating that "it would not be surprising if internal motions had been subjected to selective pressure during evolution. Just as structure is selected on the basis of function, there could be selection for certain internal motions, a consequence of the structure, if they had specific functional roles." Thus, thermally driven kinetic motion is an essential element of protein function, with functional selection of specific motions or forms evolving over time.

As an example of a selective form that can be influenced by magnetic fields, according to the present invention, some enzymes have ligand-bound ions that can impart stability and conformational changes necessary for reaction site to orient to optimal enzymatic activity. Frauenfelder et al (1988) note that different conformational states of a

working protein have the same overall structure and function but have varying structural details and rates at which the function is performed. Bialek et al. (1989) suggest that the most important enzyme configurations are those that reflect the optimal compromise between structures with high reaction probability and small strain energy in the protein. Specific details of the protein dynamics, particularly as they apply to functional properties of systems, remain unclear, although details of ion-enzyme interactions are being studied using synthetic peptides to provide a more explicit description of the interaction of ions and the binding sites in proteins (Regan, 1993).

However, the dynamic view of conformational states is important (Karplus et al., 1987). The interaction of an ion with its ligands in a protein can be viewed as an oscillator with a characteristic set of vibration frequencies (or, alternatively, a characteristic set of energies). Changes in protein function or enzyme activity are presumably a function of various minor structural or conformational states assumed by the protein represented by changes in energy levels of the reaction sites. Ions can have substantive roles as cofactors in establishing the appropriate conformational state or dynamic structure. Thus, thermal energy in active biological systems is normally present to promote random transitions between protein energy levels.

Changes in ion energetics caused by imposition of magnetic fields involves protein dynamics and leads to changes in enzyme kinetics. Thermal motions of solute molecules are relatively broad-band, nonspecific influences on enzyme-ionic cofactor complexes. It is possible that a critical ion may be bound in a protein cavity that shields it from collisions with solute molecules and precludes hydration of that ion. Consistent with the comments by Karplus and Petsko (1990), the present inventors have speculated that the natural vibration modes of the protein, particularly the ion cavity and the active site, may have evolved in tune with the vibrational modes to the specific ionic cofactor. It may be that these preferred vibratory modes for enzymatic activity are a

consequence of ion cofactor binding. These modes can then be altered when the protein is bound to an ion at a predetermined site. Assuming that vibrational modes in enzymes contribute to their activity, then ion cofactor binding can be used to switch the activity on or off. Thus, the action of thermal energy from solution molecules surrounding the protein may not only promote random transitions between protein energy levels, but also supply energy to specific vibratory modes in the ion-enzyme complex that are critical for enzyme activity. From this perspective, resonant interactions of magnetic fields with a critical bound ion could conceivably alter the vibrational dynamics between this ion and its protein ligands(s) by splitting and modulating the energy states of this complex. Thus, fields might sufficiently alter the spatial and temporal aspects of the vibratory interaction process, the resident times at given levels, the number of levels, or the relative occupation of different levels sufficiently to distinguish the effect from random variations in these quantities. These changes could be accomplished by exceedingly small, frequency specific amounts of energy over substantial periods of time. A change in the dynamic structure of the ionic complex could then lead to a change in the dynamic structure or the vibratory mode of the enzyme reaction site, which could lead to altered biochemical activity. This view of the dynamic interaction between proteins and ions appears to provide a critical element of magnetic field perturbations of the systems such as those described by the Edmonds (1993) and IPR models.

IPR Model Development

The IPR mathematical model used herein (Blanchard and Blackman 1994; Blanchard et al. 1994 a,b,c) predicts very distinct responses that are consequences of multiple, independent variables of exposure including the DC, or static, magnetic field flux density as well as the AC magnetic field flux density, frequency, and relative orientation to the DC magnetic field.

There is support from a variety of sources for considering the influence of each of these parameters on

biological systems, but the IPR mathematical model is the first to assemble them in a coherent, experimentally accessible manner and to provide a clear indication of the expected magnitude of result, relative to that of an unexposed sample, for any given combination of the independent variables. The IPR model, in its simplest form, assumes an effect on an enzyme that is complexed with an ionic cofactor to perform its catalytic function in some reaction pathway. Magnetic fields, under conditions described by the IPR model, cause minor but significant changes in the ionic interaction with the enzyme that can alter its rate of reaction. This altered reaction rate can have biological consequences for the whole cell, and consequently for the organisms.

The IPR mathematical model is based upon an earlier derivation of the influence of parallel exogenous AC and DC magnetic fields at the atomic level by Podgorestkii and Khrustalev (1964). Podgoretskii's derivation for atomic spectroscopy was extended to biological systems by Lednev (1991). Lednev's model contained some critical mathematical errors and focussed strictly on a limited set of ions that could be bound to the Ca^{++} binding protein. The present inventors have corrected the errors in the Lednev model, extended the set of ions potentially influenced by magnetic fields, and described the expected response form when the energy levels of two or more resonant ions are altered by external magnetic fields.

As originally formulated by Podgorestkii, an external DC magnetic field creates a Zeeman splitting of the quantum energy levels of each ion. These split energy levels are then frequency modulated by an external AC magnetic field. The involvement of frequency modulation suggests that the IPR response is distinct from the random effects of amplitude modulated thermal noise. Frequency modulation does not require addition of kinetic energy to the system. Rather, frequency modulation locally alters the potential energy of the system. The IPR mathematical model indicates that, although the potential energy alteration may be small on the global scale (and certainly less than the overall thermal noise level), the

resultant small changes created locally in the population distributions may be significant in producing specific biological effects if associated with an ion resonance.

A fundamental parameter of the IPR mathematical model is the frequency index, which is the ratio of the ion's characteristic resonant frequency, f_c, to the frequency of the AC magnetic field oriented parallel to the DC magnetic field, f_{ac}:

$$n = f_c / f_{ac} \qquad (1)$$

where the frequency f_c is coincidentally the same as the cyclotron resonance frequency, involving the ratio of an ion's charge (q) to its mass(m), or

$$f_c = qB_{dc}/2\pi m \qquad (2)$$

Here we rename f_c the characteristic resonant frequency in order to avoid confusion with Liboff's ion cyclotron resonance models. Within the IPR model, the critical term is n, describing the key relationship between system specific (ion) parameters (q/m) and externally imposed conditions (f_{ac} and B_{dc}). Note that IPR model harmonics (n = 1, 2, 3, ...) are inverse to harmonics described by the ICR model (discussed below). In the IPR mathematical model, n defines an ion resonance condition associated with a specific splitting of an energy level, arising from the applied DC magnetic field.

The IPR mathematical model examines how the probability of ion transitions to lower energy levels changes when the ion is near resonance. According to the IPR model, the probability of ion transition, p, is given by the equation

$$p = K_1 + K_2 \times (-1)^n \times J_n(n \times 2 \times B_{ac}/B_{dc}) \qquad (3)$$

only when the ion's frequency index is integer valued (J_n is the Bessel function of the first kind, order n; B_{ac} is the peak AC flux density; and B_{dc} is the DC flux density). K_1 is the response of the system when $B_{ac} = 0$ and K_2 is a real constant whose value depends on the particular ions contribution to the biological endpoint measured. The IPR mathematical model predicts that for ions with non-integer frequency indices, p will equal K_1 (a constant that is

independent of B_{ac}).

Essentially, the IPR mathematical model predicts that when the applied DC field and AC frequency create a resonant environment for an ion, the probability of transitions between energy states associated with that ion will be modified in a deterministic way. The modification for that ion is proportional to a Bessel function whose order is selected by the ion's integer-valued frequency index. Whether the contribution from the Bessel function is additive or subtractive, at least at the atomic level, is also determined by that ratio, with odd integer values for the ratio inverting the sign of the Bessel function because of the $(-1)^n$ term. This distinction is expected to be significant primarily at the molecular level since at more complex levels (cellular, organ, or organism), molecular actions may reinforce or restrain the selected biological/chemical endpoint.

There is a distinction in the treatment of harmonics between the ICR and the IPR mathematical models. The ICR model postulates resonance-type effects when the AC frequency is some integer multiple of the fundamental ICR frequency ($f_{ac} = k \times f_c$ where k= any integer and f_c = fundamental ICR frequency). By contrast, the IPR model derives a relationship between the fundamental ICR frequency, f_c, and integer multiples of the applied AC frequency that is the inverse of ICR model harmonics. A special case occurs at $\underline{n}$=1, where the IPR predicted resonance is also an ICR mathematical model resonance.

The IPR model is further distinguished from the work of Liboff et al. by its continuous predictions (mathematical function) of distinct response differences from the non-exposed state as B_{ac} is increased. Aside from an ambiguous postulation of effect at $B_{ac} = B_{dc}$ (where B_{ac} is interpreted as peak or rms value by different authors), the ICR model is mute on this issue.

Equation 3 differs from the original Lednev formulation in two critical ways: There is an additional $(-1)^n$ term on the right side of the equation, and the argument to the Bessel function contains an additional factor of 2.

Although the Lednev model focused on magnetic field effects on calcium binding proteins, the IPR model does not require or exclude calcium except by its resonance characteristics. The IPR model explicitly recognizes that a system's response may reflect the combined influence of several different near resonance ions. In the absence of contrary information, ions are assumed to act independently to produce the observed response, and the IPR model predicts that the response will be a linear sum of the individual response functions uniquely characteristic of the ions within the system.

In applying the IPR mathematical model to complex biological systems, the ions at resonance are assumed to be in the unhydrated state. This situation may be found, for example, when transition metal ions are loosely bound by ligands in a molecular structure. Table 1 lists biologically significant ions (compiled from Liboff, 1985, 1992; Liboff and Parkinson, 1991; EPRI, 1990; Abrams and Murrer, 1993; Karlin, 1993; Lippard, 1993; O'Halloran, 1993; Pyle, 1993; Regan, 1993; Thomas et al., 1986) for which IPR model predictions have been made, and shows how the frequency index for each ion changes with variations in either the flux density of the DC magnetic field or the AC magnetic field frequency. This table is not an all inclusive list. Appearance of an ion in Table 1 indicates a potential biological role but does not imply significant activity of any particular ion in a given system. To maximize the number of possible options, the present inventors have included all valences and have not limited the selection to the oxidation states normally considered biologically relevant, as these may exist momentarily in some biological/chemical systems as intermediate states and could be affected by the imposition of a magnetic field. In addition, the masses used are for unhydrated ions, as required by the model and supported by the work of Chiabrera and Bianco (1991); otherwise, the effective ionic mass could be infinitely variable.

Table 1: Frequency Indices for Various Potential Biologically Significant Ions

Ion Name & Valence	q/m C/kg * 10^6	f_{ac} = 45 Hz B_{dc} = 370 mG	f_{ac} = 25 Hz B_{dc} = 205.5 mG	f_{ac} = 45 Hz B_{dc} = 575 mG	f_{ac} = 45 Hz B_{dc} = 20 mG
Lead 2	0.925	0.121	0.121	0.188	0.007
Barium 2	1.395	0.183	0.183	0.284	0.010
Copper 1	1.510	0.198	0.198	0.307	0.011
Cadmium 2	1.700	0.222	0.222	0.346	0.012
Lead 4	1.850	0.242	0.242	0.376	0.013
Strontium 2	2.187	0.286	0.286	0.445	0.015
Potassium 1	2.450	0.321	0.321	0.498	0.017
Chlorine 1	2.720	0.356	0.356	0.553	0.019
Zinc 2	2.940	0.385	0.385	0.598	0.021
Copper 2	3.020	0.395	0.395	0.614	0.021
Cobalt 2	3.250	0.425	0.425	0.661	0.023
Nickel 2	3.260	0.427	0.426	0.663	0.023
Iron 2	3.430	0.449	0.449	0.698	0.024
Manganese 2	3.490	0.457	0.457	0.710	0.025
Chromium 2	3.680	0.482	0.481	0.748	0.026
Vanadium 2	3.762	0.492	0.492	0.765	0.027
Arsenic 3	3.836	0.502	0.502	0.780	0.027
Sodium 1	4.180	0.547	0.547	0.850	0.030
Calcium (45) 2	4.290	0.561	0.561	0.872	0.030
Calcium (40) 2	4.780	0.626	0.625	0.972	0.034
Cobalt 3	4.880	0.639	0.638	0.992	0.035
Nickel 3	4.900	0.641	0.641	0.996	0.035
Iron 3	5.150	0.674	0.674	1.047	0.036
Manganese 3	5.230	0.684	0.684	1.064	0.037
Chromium3	5.530	0.724	0.723	1.125	0.039
Vanadium 3	5.642	0.738	0.738	1.147	0.040
Molybdenum 6	5.992	0.784	0.784	1.219	0.042
Arsenic 5	6.394	0.837	0.836	1.300	0.045
Manganese 4	6.980	0.913	0.913	1.419	0.049
Vanadium 4	7.523	0.984	0.984	1.530	0.053
Magnesium 2	7.890	1.032	1.032	1.605	0.056
Vanadium 5	9.404	1.231	1.230	1.912	0.067
Manganese 6	10.500	1.374	1.374	2.135	0.074
Chromium 6	11.100	1.453	1.452	2.257	0.079
Sulphur 4	11.954	1.564	1.564	2.431	0.085
Manganese 7	12.200	1.597	1.596	2.481	0.086
Lithium 1	13.800	1.806	1.805	2.806	0.098
Sulphur 6	17.930	2.346	2.346	3.646	0.127
Hydrogen 1	95.600	12.510	12.507	19.442	0.676

For example, allowing a ± 10% resonance bandwidth, the near-integer-valued frequency indices, n, when B_{ac} = 370 mG and f_{ac} = 45 Hz are 1 (Mn 4, V4, and Mg 2) 2, (Li) and 12 (hydrogen). For B_{dc} = 575 mG and f_{ac} = 45 Hz, the possible frequency indices are 1 (Ca2, Co3, Ni3, Fe3, Mn3), 3(Li), and 19 (hydrogen). According to the IPR mathematical model, variations in B_{dc} or in f_{ac} individually will change the ions for which frequency indices are near integer value, while proportional changes in B_{dc} and f_{ac} will maintain the selection. For example, the ratio of 370 to 45 is the same as the ratio of 205.5 to 25, so the frequency indices and resonant ions for those two combinations are identical. Since the mathematical model is selective for ions solely on the basis of their charge to mass ratio, effects on ions with exactly the same frequency index will be indistinguishable except if the measured endpoint is known to be influenced by a particular ion. Ions with closely related frequency indices at given values of B_{dc} and f_{ac} can most easily be distinguished at higher B_{ac} flux densities.

Effects predicted by the IPR mathematical model are most easily tested when exposure values create resonance conditions for either a single biologically significant ion or only one near-integer-valued frequency index. When the exposure values create resonance conditions for a variety of ions representing several frequency indices, the resulting response function may become quite complex, requiring an extensive number of exposure test points to sample that response function unambiguously.

The enhanced sensitivity predicted by the IPR mathematical model for small changes in B_{ac} as B_{dc} approaches zero is limited by the finite bandwidth of ionic resonances.

Control of the AC frequency and the DC field strength so as to create a resonance for an ion suspected to be active in the creation of said biological/chemical action within the biological/chemical system is such that the ratio of the ion's charge to its mass is an integer multiple of the ratio of the angular frequency of the AC magnetic field ($2*pi*f_{ac}$) to the flux density of the DC magnetic field. The resonance claimed

here is the mathematical inverse of the Ion Cyclotron Resonance already addressed by Liboff et al. Therefore they are the same ONLY when the integer value of the present claim is uniquely one. Further, the ions considered by ICR are a subset of those specified by IPR. An ion may be suspected to be active as a result of previous research (see for example the list of ions identified through other studies as having potential biological significance given as Table 1 of this application), or it may be identified as a result of testing for other ions (see for example the work of Blackman et al., 1994, that identified hydrogen as a potentially significant ion and the subsequent work by Trillo et al., 1994, confirming a biological role in accordance with the claims of this application).

IPR Mathematical Model Application

Although there are three apparently independent variables in the IPR mathematical model, they are coupled within the model, so tests to demonstrate clear consistency with IPR model prediction must consider the interrelated nature of the variables. The basic tests are described below, beginning with variations in B_{ac}, to determine whether the response observed in a particular biological system is consistent with the predictions of this IPR model.

In any test or use of the IPR model, it is critical that the magnetic fields to which the biological systems are exposed are only those required by the model. Thus it is essential that the nonparallel component of the DC magnetic field be reduced to as close to zero as possible.

Changes in B_{ac}

B_{ac} is the only variable unrelated to ion selection, because it appears only in the argument of the Bessel functions. Thus a test that examines a range of B_{ac} exposures while maintaining B_{dc} and f_{ac} constant provides a quick test of IPR applicability. The f_{ac} and B_{dc} values should be selected so that at least one ion that is expected to be active in the system under test has a near-integer-valued frequency index. Multiple test points are generated by varying B_{ac} such that the tests examine exposures for which $\underline{n} \times 2B_{ac}/B_{dc}$ varies from near

zero, i.e., B_{ac} = zero, to at least the first zero crossing of the least-order-selected Bessel function (i.e., the least-valued near-integer frequency index, determined by the selected values of B_{dc} and f_{ac}). For example, if the least valued near integer frequency index is 1 ($\underline{n}$ = 1) then the least-order Bessel function selected is J_1 and the argument range over which one would observe an effect increasing to a maximum and then returning to no effect would be $\underline{n} \times 2B_{ac}/B_{dc}$ = 0 - 3.8. The predicted system response over this range, assuming either a minimum of other near-integer frequency indices, or dominance of the J_1 based response, is approximately U-shaped (see Figure 5a). It should be noted that the value of K_1 (determined by the sham response for no AC field exposure) sets the starting point and the value for K_2 varies the depth of variation for the response associated with ion(s) having a common near-integer-valued frequency index (equation 3), but neither coefficient changes the relative positions of the minima, maxima, or "zero crossings" (null effect relative to unexposed system) of the predicted IPR mathematical model response function. In fact, since the value of K_1 is established directly by control data, there is only one variable (K_2) per frequency index that can be adjusted to fit the acquired data. When B_{dc} and f_{ac} are selected such that no ion is at resonance, changes in B_{ac} are not predicted to alter the observed response, as discussed above and in the next section.

Changes in B_{dc}

Varying B_{dc} by itself would bring different ions on and off resonance, changing their frequency indices in the process. Since the IPR mathematical model predicts a flat response (p = constant) when no ion is at resonance, detuning for all ions through an appropriate selection of the DC flux density tests the IPR model while eliminating one of the variables in which B_{dc} plays a role (no Bessel function is selected, so there is no influence on the argument). This provides a second critical test of IPR model applicability. Hydrogen provides the limiting case: its charge to mass ratio is the largest possible of all elements and it is a potential biologically significant ion. For the "off-resonance" test,

B_{dc} is chosen and fixed such that the frequency index for hydrogen is well below unity, and the same f_{ac} used in the previous test is maintained in this test. Given these fixed parameters, test points should be generated to test the system response over a range of Bessel function arguments ($\underline{n} \times 2 \times B_{ac}/B_{dc}$) comparable to those tested in the previous case. The predicted system response under these conditions is flat, with no variation across the entire range of AC field values tested (see Figure 5b).

Changes in f_{ac}

Finally, the effects observed in the preceding tests must be consistent across a variety of AC frequencies. Variations strictly in the AC field frequency, f_{ac}, would change two components of the IPR model: the near-integer frequency indices and the argument to the selected Bessel functions. If only f_{ac} were varied during such tests, the results would be complicated to interpret in terms of the IPR model, because it would change the frequency index for each ion, could select a different Bessel function, and would form a different Bessel function argument. A cleaner test examines the consistency of response when both the AC frequency and the DC flux density change proportionally to maintain the frequency indices selected in the first test. This is critical, in that the effects observed in the earlier tests are specifically a function of the active ions selected. Test points should then be generated to give the same argument to the Bessel function(s) as in the first test. In essence, this performs the first test at a different frequency. The predicted response function is the same as that predicted for the first test (e.g., U-shaped if the J_1 term is dominant).

Using the three critical tests described above, one skilled in the art can readily identify whether a biological system's response to parallel AC and DC magnetic fields is consistent with the IPR mathematical model. These tests are based upon controlled variations in three basic parameters: the flux densities of parallel components of the AC and DC magnetic fields and the AC magnetic field frequency to examine on and off resonance cases. For "on resonance:" tests, the response

forms are distinctly nonlinear. The "off resonance" response is predicted to be flat across a range of AC flux densities, in stark contrast to the "on resonance" case. Once a system is shown to respond consistently with IPR model predictions, as described above, clear and distinct predictions can be made about its expected response to different values of B_{ac}, f_{ac} or B_{dc}.

Empirical Test of IPR Model Interactions

The response of PC-12 cells under the exposure conditions suggested is described below, and finds clear consistency with the IPR model. The following description is one of many possible embodiments of the present invention by which the IPR model can be shown to predict a biological response to controlled parallel AC and DC magnetic fields. This description is included merely for illustration, and not for limitation. The predictive nature of the model is discussed after the detailed demonstration of the IPR model consistency is established.

The PC-12 cell line was used for these experiments. The neurite outgrowth (NO) these cells display in response to stimulation by nerve growth factor (NGF) has served as a basis world-wide for investigations of NGF-induced changes in nervous system-derived cells (Levi et al., 1988) since Greene and Tischler (1976) first established the line from a rat adrenal pheochromocytoma. The PC-12 assay system has been shown previously to be differentially responsive to AC magnetic fields. The assay is simple, well established, and widely used among those skilled in the art of neuroscience. Because this system examines functions at the isolated, single-cell level, its simplicity is of considerable advantage to theoreticians studying the interactions of electromagnetic fields with biological systems. This neurite outgrowth assay system is currently used to evaluate neurotransmitted production and second messenger signaling processes and subsequent genomic events, particularly those induced by nerve growth factor stimulation. Earlier tests demonstrated that NGF-stimulated NO in PC-12 cells could be inhibited by exposure to 50 Hz magnetic

fields during a 22 hour treatment period (Blackman et al., 1993). The response demonstrated a magnetic field strength dependence across the range of 35-90 mG(rms) {3.5 and 9.0 μT (rms)}. The inhibition was further shown to be independent of any induced electric field. In addition, there was an observed dose rate dependence of this inhibition over the frequency range of 15-70 Hz. These exposures consisted of a vertical AC magnetic field with an ambient static DC magnetic field, wherein the DC field contained both vertical and horizontal components. At the time, there was no apparent explanation for the observed changes in NO in PC-12 test cells.

Materials and Methods

Growth and Preparation of Cells

The PC-12 cells used in these experiments were obtained from the Tissue Culture Facility at the University of North Carolina at Chapel Hill. The cells were primed by growth in RPMI 1640 medium (Gibco 320-1875), supplemented with 10% horse serum (Gibco 200-650), and 5% fetal calf serum (Gibco 240-6000), and 100 units/ml each of penicillin and streptomycin on six-well collagen-coated plates (Costar 3046) in a 5% CO_2 incubator at 37°C, with addition of NGF (50 ng/ml, 2.5 S, Sigma 6009) at plating and every other day for six days. On day 7, the medium was removed from the primed cells and the cells were washed three times with complete medium to remove any remaining NGF. The cells were then removed from the plates by trituration, centrifuged, resuspended, counted, placed into 10% dimethylsulfoxide (DMSO), frozen, and stored at -80°C in 1 ml volumes of 1 x 10^6 cells/ml. Prior to each experiment, the cells were thawed and rinsed with medium three times, and the contents of one ampoule were placed into 50 ml of medium.

The collagen-coated petri dishes were prepared by placing 0.45 ml of stock solution {5 mg of collagen (Sigma rat tail type VII 8897) in 125 ml of 0.1 M acetic acid} on 60 mm dishes (Costar 3060). The dishes were then air dried in a sterile hood. Before use, the dishes were rinsed with medium to neutralize the pH. Five milliliter volumes of primed cells, at 2 x 10^4 cells/ml, were plated onto 60 mm collagen-coated

petri dishes. At this plating density, the cells covered less than 10% of the growth surface.

The cell medium was supplemented with 5 ng/ml of NGF (Sigma 6009) for all but the zero NGF control. This NGF concentration had previously been shown to induce NO in approximately 50% of the cells (Blackman et al., 1993). All dishes in the exposure system contained 5 ng/ml of NGF. To establish the control parameters in each experiment, two dishes with and two dishes without NGF were placed within the control (nonexposed) area in the same incubator. The one exception to this occurred for one trial each of tests 1 and 2, when only one dish was used for each control condition.

Magnetic Field Exposure System

The PC-12 cells were exposed to prescribed sets of parallel AC and DC magnetic fields while housed in a 5% CO_2 incubator maintained at 37°C. Prior to testing, the ambient AC and DC fields were measured with a Bartington MAG-03 fluxgate magnetometer. The ambient DC magnetic field within the exposure apparatus was 447 mG at an inclination of 60°N (389 mG vertical and 220 mG horizontal). The ambient 60 Hz magnetic field in the exposure apparatus was 8.8 mG(rms) {7.0 mG(rms) vertical and 5.3 mG(rms) horizontal}, whereas within the shielded area it was 1.2 mG(rms) {0.74 mG(rms) vertical and 0.92 mG(rms)horizontal}.

The following description is one of many possible embodiments of the present invention by which controlled magnetic field exposures can be created. This description is included merely for illustration, and not for limitation.

A pair of Helmholtz coils, consisting of two 100-turn, 20-cm. diameter coils of enameled wire (22 awg; 35 ohms resistance per coil), aligned coaxially 10 cm apart, were oriented and energized to control the vertical DC and AC magnetic fields (cf. Blackman et al., 1994). As needed, both coils were energized with a direct current to adjust the ambient, vertical DC magnetic field in the sample area. Only the lower coil was energized with AC current to create sinusoidal magnetic fields of decreasing strength on the coil axis as a function of distance above the coil. The six PC-12

samples to be exposed were placed coaxially with the coil center line. In some cases a seventh dish was added within the stack to provide the desired range or spacing of B_{ac}. To reduce the horizontal components of the ambient DC magnetic fields to values as low as possible (<2mG), two square coils, 27 cm on a side and separated by 17 cm wound with 200 turns of enameled wire, were included as part of the exposure system.

The exposure system, consisting of the coil systems and cell samples, was located on a plastic shelf in the upper two-thirds of the incubator space. A Co-netic metal magnetic-field shield (Magnetic Shield Corp.) created a shielded area, also within the incubator, to serve as a control, or unexposed, area. This shield reduced the flux density of the magnetic field generated by the exposure system to less than 1% of ambient (cf. Blackman et al., 1994). The Co-netic shield was in a tube configuration position near the bottom of the incubator space, with the long axis fore and aft to allow for air circulation and ease of positioning the dishes. No temperature differences greater than 0.1°C (resolution of meter) were observed using Chromel-Alumel thermocouples between different samples in the coils, with the coils energized at the highest currents used. No temperature differences were observed between the exposed samples and the shielded samples. Sham exposures revealed no difference in cell response between exposure and shielded positions.

A function generator and an ampmeter, connected in series with the lower circular coil, produced and monitored the sine wave current and, as needed, the desired direct current. Measurements with a frequency meter verified the proper setting on the function generator. All generating and monitoring equipment was located outside the incubator.

The AC magnetic fields cited in these experiments were verified at the locations of each dish with a calibrated gaussmeter (Bell 640) and Hall effect probe. When the coils were placed in the CO_2 incubator, the proximity of the inner walls reduced the actual flux density at the test specimen locations by approximately 20% from the calculated values. The measurements reflect this reduction, and are cited as root-

mean-square (rms) values read from the gaussmeter, with their corresponding peak (pk) equivalents given in parentheses.

Exposure

The PC-12 cells were exposed for 23 hours, beginning within three minutes after plating, to examine responses to four distinct tests, shown in Table 2. (Note that these conditions did not tune for calcium ions.) The observed effects were both consistent with the predictions of the IPR mathematical model and confirmed the validity of extending the ion list beyond calcium. Additionally, the repeatability of the results was checked by running tests 1, 3 and 4 three separate times and tuning test 2 four times. For every exposure, two sets of controls were placed in the shielded area: one set with and one set without NGF.

Table 2. Exposure Conditions

Critical Quantity	*Units*	*Test 1*	*Test 2*	*Test 3*	*Test 4*
AC frequency	Hz	45	45	45	25
B_{dc} - horizontal ($B_{dc}H$)	mG	< 2.0	< 2.0	< 2.0	< 2.0
B_{dc} - vertical ($B_{dc}V$)	mG	366	366	20	203
B_{ac} - vertical (min.)	mG rms (pk)	77 (108)	200 (284)	7.9 (11)	78 (110)
B_{ac} - vertical (max.)	mG rms (pk)	200 (283)	468 (662)	21 (29)	181 (256)
$B_{ac}V$(rms)/$B_{dc}V$ range	---	0.21 - 0.55	0.55 - 1.28	0.40 - 1.03	0.30-0.89
$B_{ac}V$(pk)/$B_{dc}V$ range	---	0.30 - 0.78	0.78 - 1.81	0.54 - 1.41	0.54-1.26

A common reference point was selected, consisting of an AC frequency of 45 Hz for tests 1-3, $B_{dc}V$ of 366 mG(ambient) for tests 1 and 2, and a horizontal DC flux density ($B_{dc}H$) reduced to as close to 0 mG as possible (less than 2 mG in all experiments). All ions from Table 1 that were close to resonance for B_{dc} = 366 mG and B_{ac} at 45 Hz, and two ions that are far from resonance for these exposure conditions, are shown in Table 3, where n is the frequency index defined earlier. For test 4, the ratio of B_{dc} to f_{ac} is the same as for tests 1

and 2, so that Table 3 remains applicable. Test 3 was designed specifically for off-resonance conditions for all ions listed in Table 1.

Table 3. Ions Near and Off Resonance.

Ion	*Frequency Index, n*
Ca(2)	0.619
Fe(3)	0.667
Mn(4)	0.904
V (4)	0.974
Mg(2)	1.021
Li(1)	1.786
H(1)	12.375

In this example, Mn(4), V(4), Mg(2), Li(1), and H(1) are within 10% of an integer-valued frequency index. By contrast, Ca(2) and Fe(3) are well off-resonance. Note that, as either the DC field flux density or the AC field frequency changes, the number and closeness of ions to resonance conditions will vary. A more complete list of potentially biologically significant ions is given in Table 1 of this application.

Under the exposure conditions used in tests 1, 2 and 4, at least three ions from Table 1, manganese, vanadium, and magnesium, were within 10% of their predicted resonance peak for n = 1. Although there is no *a priori* way of knowing that these ions are present or active in PC-12 cell generation of neurites, the fact that Landreth et al. (1990) and Sano (1992) identified magnesium and manganese as critical cofactors in the phosphorylation of microtubule-associated proteins in neurites that are induced by NGF stimulation suggests an enhanced possibility that an effect might be seen under the prescribed

exposure conditions. In addition, at least three potentially significant ions are near resonance, two of which are Mg and Mn, had the same frequency index. Two other potential biologically effective ions, calcium and iron, were far from resonance under these conditions. Lithium, with a frequency index near 2, is a candidate for effective ions whose frequencies can be altered by imposition of a magnetic field.

Different biological outcomes can be expected to exhibit different sensitivities to various ions.

Using the techniques described herein, one can establish more clearly which ions and oxidation states are relevant and identifiable with this technique for particular biological systems. The critical influence ions have on the structure and function of biological molecules was recently highlighted in review of the emerging area of bioinorganic chemistry (Lippard, 1993; Karlin, 1993; Pyle, 1993; O'Halloran, 1993; Abrams and Murrer, 1993; Regan, 1993). These reviews support the hypothesis that the fit of the IPR model to the experimental data may consider one or all of the $\underline{n}$=1 ions, magnesium, vanadium, and manganese; the $\underline{n}$=2 ion lithium; and the $\underline{n}$=12 ion, hydrogen, as involved in the field-induced inhibition of neurite outgrowth.

The influence of ions involved in neurite outgrowth or other biological/chemical changes may involve at least one or more other critical biochemical steps. It is possible that some of the molecular-level actions could oppose each other so that observed biological responses, in contrast to molecular-level responses, may in some instances be small because of potential offsetting influences of different ions. For example, the analysis below suggests that lithium may play a minor opposition role vs. that of the other ions. Because, under the exposure conditions selected from these tests, the frequency index of 1 is common to magnesium, vanadium, and manganese, it is more difficult to determine directly exactly which molecular processes, e.g., enzyme reactions, are affected in the exposures. Nevertheless, examination of exact conditions for predicted null responses for each of these $\underline{n}$ = 1 ions at larger values of B_{ac} (or smaller values of f_{ac} and B_{dc})

can provide the information to select the affected ion(s) and thus ultimately provide identification of the molecular processes involved.

Assay Procedures

After exposure, the cells were assayed in a blinded fashion from pseudo-random (i.e., non-overlapping) areas near the center of each dish. The neurite outgrowth assays determined the number of cells either with a neurite length greater than the cell body or with neurites containing either a branch or a growth cone (these accounted for approximately 5% of the total cells scored positive) to create a raw cell scoring. All cells were counted within each microscopic field, and at least 200 cells per dish were assayed.

However, each set of primed cells can produce a slightly different response to NGF. Corrections were made for differences between sets of primed cells through the responses shown by magnetically shielded controls specific to each experiment to make the measured response between test runs comparable, as described infra. These assay procedures for neurite outgrowth are well established (cf. Greene and Tischler, 1976; Greene, 1977; Blackman et al., 1993, 1994; Rukenstein and Greene, 1983).

Experimental Results

In test 1 shown in Table 2, the coil was energized with 3.9 mA(rms) to create a flux density range over which the IPR model (for n=1) predicts a monotonically decreasing probability of energy transitions between states, seen here as increased inhibition of neurite outgrowth, as the AC flux density, B_{ac}, increases. The AC magnetic field was colinear with the DC magnetic field of 366 mG. The experimentally observed decline in neurite outgrowth with increasing B_{ac} between 77 and 200 mG(rms) {108-283 mG(pk)}, shown in Figure 1, is consistent with the predictions of the IPR model. It is noteworthy that each repetition of this exposure demonstrated the same response form. The results for each run are shown to emphasize the actual ranges of the data.

Four runs of test 2 were conducted with the coil energized at 8.27 mA(rms). Again, the AC magnetic field was colinear with the DC magnetic field of 366 mG. As shown in Figure 2, the experimental results of B_{ac} from 200 to 468 mG(rms) {284-662 mG(pk)} are consistent with the predictions of the IPR model using the peak value of B_{ac}. Again, repeat tests demonstrated the consistency of this response form. A fifth run was conducted at 45 Hz under conditions {B_{ac} range of 132-344 mG(rms), or 186-486 mG(pk), generated by energizing the coil with 6.7 mA(rms)} that overlapped those in tests 1 and 2 to verify continuity of the test results across the range of AC flux densities. The results of Test 2 demonstrate the reduced effectiveness of 45Hz sinusoidal magnetic fields between 200 and 468 mG(rms) to inhibit neurite outgrowth stimulated by nerve growth factor in PC-12 cells.

Test 3 examined the response of cells over a B_{ac}/B_{dc} range of 0.54-1.41. The previous tests showed a distinctive U-shaped inhibition of neurite outgrowth as B_{ac} increased in intensity, which is consistent with IPR model predictions. However, in test 3, B_{dc} was set to 20 mG to create conditions well "off resonance" for all biologically relevant ions. As a check, it can be noted that the limiting case of hydrogen (n=0.676) is well off resonance under test 3 conditions. Because no ion is near resonance, the IPR model predicts a constant response across the AC flux density range chosen for this test {7.9-21 mG(rms), 11-29 mG(pk), 0.405 mA(rms) in the coil}. This prediction was confirmed by the empirical data shown in Figure 3, replicated three different times. As noted above, under resonance conditions, this range of B_{ac}/B_{dc} produced a U-shaped inhibition response, as shown in Figure 2.

The final test of the series evaluated whether the on-resonance results observed for tests 1 and 2 can be obtained at a different frequency. Test 4 examined the use of 25 Hz AC fields and B_{dc} = 203 mG to give on-resonance conditions identical to those in the 45 Hz cases, i.e., tests 1 and 2. In this test, the coils were energized at 3.2 mA(rms) to create a range of B_{ac} comparable to that used in tests 1 and 2. This test also examined the importance of the Bessel function

argument as the correct exposure metric. If the change in B_{dc} and f_{ac} were insignificant except for ion selection, then the exposure range would be comparable to that of test 1 and the response would be a monotonically declining % of neurite outgrowth with increasing B_{ac}. If, however, the argument of the Bessel function were the important exposure variable, then the result should be a U shape characteristic of the combination of test cases 1 and 2.

Figure 4 shows the results of three repetitions of this test in comparison to the combined results of tests 1 and 2. Figure 4a plots the data in terms of the AC flux density of B_{ac} (rms), whereas Figure 4b plots each response a a function of $2 \circ B_{ac}(pk)/B_{dc}$, as suggested by the IPR model. These results demonstrate essentially identical cellular responses as a function of exposure when compared in terms of the Bessel function argument, $2 \circ B_{ac}(pk)/B_{dc}$, indicated by the IPR model, but not when B_{ac} is used alone as the common point of reference. This result highlights the importance of using the IPR model identified form of Bessel function argument when identifying and comparing results under different exposure conditions.

Fit of IPR Model Predictions to Experimental Data

The experimental data show consistency between runs and follow the general form of response predicted by the IPR model, as demonstrated in Figure 5. Figure 5a shows the combined results of tests 1 and 2 plotted against an arbitrary fit of the n=1 prediction by the IPR mathematical model. Figure 5b demonstrates how the results of the off-resonance test (test 3) are very nearly constant across the range of B_{ac} tested. Again, the experimental data followed very closely the IPR mathematical model predictions.

It has been demonstrated above that the results of neurite outgrowth assays for PC-12 cells exposed to magnetic fields are consistent with the distinctly nonlinear and non-obvious predictions of the IPR mathematical model. The IPR mathematical model indicates how B_{dc} and the AC frequency select responses from ions based on their charge to mass ratio. The model is unique because it is the first mathematical model

to predict specific, distinct responses based on the experimentally controllable variables B_{ac}, B_{dc} and f_{ac}. The experimental results show that full characterization of the independent variables, B_{ac}, B_{dc} and AC frequency, is essential.

Figure 5a demonstrates the fit of the data with the predictions of the IPR mathematical model using just the Bessel function for $\underline{n}$ = 1 ions. Although the fit was reasonably good, the fit improved when the potential contributions from the $\underline{n}$ = 2 and 12 ions, predicted by the IPR mathematical model to be resonant under the current exposure conditions, were included (R^2 = 0.77) (see Figure 6).

Under the particular set of exposure conditions tested, a distinct, repeatable influence was observed on neurite outgrowth in PC-12 cells as a function in increasing B_{ac}. The response form was consistent with the predictions of the IPR model, with a predominant effect by $\underline{n}$= 1 ions (consisting of any or all of Mg, Mn, V). One of the crucial distinctions between the IPR model and its predecessors is the extension of proposed influence to a variety of ions beyond calcium and magnesium that have shown biological significance. It was discovered that the biological/chemical system itself determines whether any particular ion is sufficiently near resonance to create a change in the selected observable (in this case, neurite development). The exposure conditions created $\underline{n}$=1 for several biologically significant ions, including Mn^{4+}, V^{4+}, and Mg^{2+}, in addition to the $\underline{n}$=2 ions previously identified. This result is consistent with the dominance of an Mg^{2+} ion-based effect under resonance conditions recognized by all three models. More compelling evidence supporting the possible involvement of both Mg^{2+} and Mn^{4+} resonances in the neurite outgrowth response of PC-12 cells to prescribed combinations of B_{ac}, f_{ac} and B_{dc} comes from reports by Landreth et la (1990) and Sano (1992), cited by Blackman et al. (1994, p.243), identifying both magnesium and manganese as cofactors in the phosphorylation of microtubule-associated proteins in neurites that are induced by nerve growth factor.

The IPR mathematical model suggests that, as a first approximation, each ion functions independently to produce the observed response and that the overall response will be a linear, weighted sum of the individual response functions, unless there is evidence to the contrary. To examine how closely the experimental data for tests 1 and 2 were predicted by the IPR mathematical model, the normalized data were fit using the iterative multivariate secant method to determine least squares estimates of the coefficients ($K_{2,x}$) of the Bessel functions in the IPR-predicted response form corresponding to frequency indices of 1, 2 and 12:

$$\text{Fit} = 100 - |K_{2,1} \times J_1(2B_{ac}/B_{dc}) + K_{2,2} \times J(2x2B_{ac}/B_{dc}) + K_{2,12} \times J_{12}(12 \times 2B_{ac}/B_{dc})|$$

An absolute value was incorporated in the data fit because it was believed that the NGF-induced neurite outgrowth process in PC-12 cells is maximally efficient without any perturbation from fields. Under parallel AC and DC magnetic field exposure, any perturbation away from optimum response would make the induction of neurite outgrowth less efficient regardless of the molecular level direction of the perturbation or relative efficiency. This situation is not necessarily universal, as demonstrated by Ross (1990), whose assay of rabbit ligament fibroblasts showed both inhibition and proliferation of growth, depending on the exposure conditions.

The IPR mathematical model is based upon independent contributions from ions at resonance, unless there is evidence to the contrary. At low exposure values, the PC-12 cells displayed substantially less actual inhibition in neurite outgrowth than indicated by the model composed of the weighted sum of IPR mathematical model selected Bessel functions. This was confirmed by an additional set of tests at lower B_{ac} values. The data in this region show a close fit with the IPR mathematical model predictions for hydrogen alone ($\underline{n}$=12). Since hydrogen is an ion with known influence on chemical reactions through their sensitivity to pH, it was hypothesized that at lower AC field intensities, hydrogen provides sufficient stability to the biochemical structure so that the predicted magnetic field interactions with other ions would not

be observed at the level of the cell response. At higher AC magnetic field intensities there is sufficient destabilization of the biochemical molecule through field interaction with hydrogen to observe the predicted magnetic field influence on other ions complexed with that molecule. The best fit of the IPR mathematical model to the experimental data (see Figure 7) assumed an hypothesis that hydrogen has a different role, acting as a trigger ion at low field values for the PC-12 cell assay (R^2=0.94). This is not an unreasonable hypothesis, since pH plays a critical role in biological functions. If some portion of the available hydrogen in the system, meeting the criteria for the IPR mathematical model, is associated with stabilizing a site that is influenced by one of the other ions at resonance, the field-induced result may be as observed. Pilot studies that specifically isolated hydrogen as the resonant ion and tested the cell response against the predictions of the IPR mathematical model (Trillo et al., 1994) provide further support for this contention and demonstrates a clear influence of hydrogen on neurite outgrowth in PC-12 cells exposed to parallel AC and DC magnetic flux densities.

It is remarkable that the IPR mathematical model accounted for up to 94% of the variance in the PC-12 data, representing a biologically observable change, over a critical, limited, and relatively low intensity range of AC magnetic fields. The closeness of fit demonstrated between experimental data and theoretical predictions is excellent, particularly considering that the IPR mathematical model addresses field-induced changes at the molecular level whereas the experimental endpoint, neurite outgrowth, is the integrated response of an intact cell to field exposure for 23 hours.

The field-induced inhibition of neurite outgrowth under resonance conditions for Mn and Mg (n = 1) is consistent with other previously cited studies showing a critical role for Mn and Mg in nerve cell (e.g., PC-12 cell) functions. These results support the formalism of the IPR mathematical model, including the exposure-determined frequency indices, the excellent fit of the mathematical model at all B_{ac} values, the capability of the mathematical model to predict both the

relative changes in inhibition for each cycle and the intensities at which the null effects occur, and the influence of hydrogen ion resonance on the biological process, a previously unsuspected important resonant interaction.

Predictions based on IPR model and initial observations

Extended B_{ac} range

The first three tests described above were extended by exposing the PC-12 cells for 23 hours to AC magnetic fields over three higher ranges {(233-544, 430-1002, or 607-1416 mG(rms)} with a parallel DC magnetic field of 366 mG and a perpendicular magnetic field of <2mG (Blackman et al., 1995a). As with the previously described tests, the neurite outgrowth assay was used. After 23 hours' exposure to the magnetic fields, pseudo-random areas near the center of each dish were selected for cell counting. All cells within each microscope field (minimum of 200 cells per dish) were assayed in a blinded fashion. The number of cells in each dish with either a neurite length greater than the cell body, or with neurites containing either a branch or a growth cone, were tabulated as a percentage of the total number of cells examined. This raw score was normalized against the range established by the control cells with and without NGF. These assay procedures for neurite outgrowth are well established. The results displayed consistency internally between three repetitions of each test and with the earlier exposure data, where there was an overlap of AC magnetic field intensity (see Figure 8).

Extension of the IPR mathematical model to higher B_{ac} values, based on the fit to the earlier data, predicted a distinctive oscillatory type behavior, with certain exposure combinations predicted to show no variation from control values. The early data never responded more than control values, but that data was dominated by the n=1 response which predicted only an inhibition of neurite outgrowth across the narrower range of B_{ac}. Based on these results, specific exposure conditions were selected to test a broader range of B_{ac} at key points: around the minima/maxima of the extended predicted response and near predicted null responses for the

dominant response function. Since the IPR mathematical model variables (K_2 parameters) can only vary the depth of the overall response function, not the relative depths of minima/maxima n nor the B_{ac} intensity locations of null responses, the earlier data created a fixed reference prediction against which to judge the results of these extended tests.

The cell response to higher values of B_{ac} exhibited the predicted oscillatory pattern, but the observed pattern showed a distinct characteristic of the test system of the present invention: the simplest application of the IPR mathematical model predicts cycles of inhibition and enhancement (values greater than control values) of a biological response, whereas in the above-noted experiments an oscillating pattern was observed that cycled between inhibition and control values. This suggests that in the PC-12 system, the control (unexposed) values represent a maximal response of the system under the IPR model specified parallel AC and DC magnetic field exposure conditions used in these experiments.

Statistical analyses of the best fit of the data by the IPR mathematical model over the extended AC flux density range, compared against the results of the analysis over the narrower range, demonstrate that the fit of the model to the earlier data collected over the narrower range of AC flux densities is very predictive of results seen at higher values of AC flux densities. Inclusion of the "hydrogen trigger" hypothesis for low B_{ac} data does not weaken this predictive capability.

Hydrogen Resonance

Because hydrogen's charge-to-mass ratio is much larger than any other biologically significant ion, it provides a unique test case by which a single ionic bandwidth can be clearly measured. Further, hydrogen had never before been identified as a potentially resonant ion in the work of either Lednev or Liboff, so directly tuning for it would be a severe test of the IPR model's predictive capabilities. Trillo et al. (1994) have confirmed that, under IPR model conditions designed to establish a frequency index of n = 1 for hydrogen, PC-12

cells showed a pattern of neurite outgrowth inhibition consistent with IPR model predictions.

The neurite outgrowth of PC-12 cells under predicted resonance conditions for the hydrogen ion were evaluated using three different combinations of AC and DC magnetic fields. The first set of tests examined the nerve outgrowth response in cells exposed to 45 Hz magnetic field with a B_{ac} ranging from 2.9 to 41.1 mG (rms) in the presence of DC magnetic field of 29.6 mG, over which a U-shaped resonance was obtained.

A second experiment was conducted to create "off-resonance" conditions for all ions using a 45 Hz magnetic field, 7.9 to 20.5 mG (rms) B_{ac} and 19.7 mG B_{dc} control.

A third experiment was conducted in which the B_{ac} and B_{dc} values were the same as in the off-resonance experiment, and the field conditions were tuned for resonance of the hydrogen ion by changing the AC frequency to 30 Hz.

Under magnetic field conditions in experiments 1 and 3, the IPR model predicts a "U-shaped" curve of inhibition of nerve outgrowth as a function of the values of B_{ac}. After a 23-hour incubation and exposure to a magnetic field in the presence of nerve growth factor, the neurite outgrowth was analyzed. The results showed that the nerve growth factor stimulation of neurite outgrowth in PC-12 cells was affected as predicted under field conditions in experiments 1 and 3, but not under the conditions of experiment 2, the off-resonance condition. The values of neurite outgrowth fit the predictions for the IPR model ($R^2=0.85$) except for a short range of B_{ac} between 1.5 and 2.1 mG (rms) which, according to the model, are expected to provoke the maximal response. The apparent lack of response to specific B_{ac} values over this range suggests that an additional, perhaps hydrogen specific, mechanism could be involved in the particular response of the PC-12 system.

A subsequent test exposed NGF-stimulated PC-12 cells to magnetic fields tuned for near hydrogen resonance, to test the hypothesis of a $\pm$ 10% ionic bandwidth. The center frequency was 45 Hz, 42.5 and 47.5 Hz were postulated as being near resonance, and 40 and 50 Hz were postulated as frequencies bounding the assumed $\pm$ 10% resonance bandwidth. Each exposure

condition test was repeated three times. The results (Blackman et al., 1994) obtained at 45 Hz replicated the results obtained independently by Trillo et al. (1994), and the 42.5 and 47.5 Hz results demonstrated slightly off-resonance responses. The 40 and 50 Hz results displayed near control cell or off-resonance responses. These results support the hypothesis that resonance conditions can exist within ± 10% of the center frequency.

Influence of the perpendicular component of B_{dc} on cell response

The preparation of PC-12 cells for exposure to magnetic fields was conducted as described above, with one exception. In addition to experiments exposing six dishes of cells simultaneously, in one set of experiments only three dishes were exposed.

As described above the exposure system was housed in a cell culture incubator. The AC and DC fields were verified at the sample locations with a Bartington MAG-03 fluxgate magnetometer and representative values are given. The ambient DC magnetic field within the exposure apparatus was 387 mG at an inclination of 58°N (327 mG vertical and 207 mG horizontal). The ambient 60-Hz magnetic field in the exposure apparatus was 0.86 mG rms (0.50 mG rms vertical and 0.70 mG rms horizontal). In the control incubator, the ambient DC magnetic field was 298 mG at an inclination of 82°N (295 mG vertical and 44.1 mG horizontal). The ambient 60-Hz magnetic field was 0.84 mG rms (0.59 mG rms vertical and 0.60 mG rms horizontal). Comparison experiments between the two incubators showed no difference in cell response, measured as the percent of cells displaying neurite outgrowth, when the coils were not energized.

The tests described above included the response of the cells exposed to six flux densities of 45 Hz fields over the range 132-344 mG rms, with the B_{dc} perpendicular <2mG/parallel 366 mG. Two additional tests were conducted under these B_{dc} exposure conditions (designated V, for vertical field). Other flux densities and alignments of B_{dc} with the AC field were then tested directly at least three independent

times, using B_{dc}:

(1) perpendicular 366 mG/parallel <2mG (designated H, for horizontal field);

(2) perpendicular 366 mG/parallel 366 mG (designated H & V); and

(3) perpendicular 160 mG/parallel 366 mG (designated 0.4H & V).

The design of these experiments is a split-plot with replications as whole plots and dishes as sub plots, and was analyzed by an appropriate analysis of variance to compare H versus V, H versus H&V, H&V versus 0.4H &V, 0.4H versus V and H&V versus V. In addition, at each B_{ac} value in the H exposure test, a test was made of the hypothesis that the mean % neurite outgrowth was equal to 100. To test directly the influence of case H, B_{dc} perpendicular 366 mG/parallel<2mG, four additional runs were conducted, each using three columns of stacked empty dishes with cells located at the desired height to be exposed to 202 mG rms. These results were analyzed by a t-test that compared the response to the control response of 100% neurite outgrowth.

The neurite outgrowth assay used in the above-described experiments was used. These assay procedures for neurite outgrowth are well established (Greene and Tischler, 1976; Greene, 1977; Blackman et al., 1993, 1994). The results displayed internal consistency between the three to five repetitions of each test. Further, they were consistent with the earlier data where there was an overlap of AC magnetic field intensity.

Results and Data Analysis

The cell response to 45 Hz magnetic fields as shown in Figure 11 for various flux densities of the perpendicular and parallel components of the DC field. Case H is for perpendicular 366 mG/parallel <2mG DC fields. Case V is for perpendicular <2mG/parallel 366 mG fields. Case H&V is for perpendicular 366 mG/parallel <366 mG. Case 0.4H&V is for perpendicular 160 mG/parallel 366 mG. It is apparent from case H compared to case H&V that the perpendicular component of the

DC magnetic field dominates the cell response. The analysis of variance of the experimental design is shown in Table 4. The results of the five paired comparisons are given under H&V interaction since they are part of that interaction. All five comparisons in Table 4 are statistically different ($p<0.001$). The results of the statistical test indicate that the response of cells under each DC field exposure conditions was distinct from the others. In the H exposure case, the test of the cell response at each exposure compared to 100% was severely restricted because the number of hypotheses tested required a Bonferroni correction to be used. Under these conditions, only the results at 132, 297 and 344 mG rms were statistically different from 100%.

In order to test whether the cell response for exposure to 45 Hz, 202 mG rms case H-perpendicular 366 mG/parallel <2mG- was different from control values, an additional four tests were conducted under those conditions. The analysis demonstrated that this field condition caused a statistically significant enhancement of the cell response (118.2% NO ± 0.4 SE, n=12) over the control value of 100% NO.

The above results unequivocally demonstrate that perpendicular AC and DC magnetic fields cause changes in biological response that are different from those caused by parallel AC and DC magnetic fields. Under the exposure conditions used in this study, the parallel fields inhibit the neurite outgrowth response, whereas perpendicular field enhance the response compared, in each case, with the response observed of the unexposed cells. The cell response to parallel fields was found to be in remarkable agreement with predictions of the IPR model described above. The results with perpendicular AC and DC magnetic fields are consistent with the results observed for field-induced changes in calcium ion efflux from brain tissue in vitro observed using 315 Hz AC at 0.6 mG rms and B_{dc} perpendicular at 380 mG, parallel ~0mG. Although there is presently no comparable predictive model for perpendicular fields, it appears that one of the mechanisms responsible for the change in efflux is a magnetic resonance-like process. Further, the multiple "windows" of response to B_{ac} intensities

observed in the calcium efflux studies (Blackman et al., 1982, 1989) may be consistent with the cell response as a function of B_{ac} flux density observed here. It appears possible that a process similar to the one hypothesized for calcium efflux (Blackman et al., 1988, 1989) may have a role in these studies with nerve growth factor-stimulated neurite outgrowth in PC-12 cells as well, when AC and DC fields are perpendicular.

This demonstration of differential and even antagonistic action evoked by the parallel versus perpendicular DC magnetic fields is consistent with the different response of neurite outgrowth at 45 Hz as a function of B_{ac} intensity shown for exposures of only parallel DC fields (Blackman et al., 1994, 1995a,b) compared to exposures with both perpendicular and parallel DC fields (Blackman et al., 1995b). There appear to be at least two mechanisms by which AC and DC magnetic fields can cause changes in biological systems as exemplified by the changes induced in nerve growth factor-stimulated neurite outgrowth in PC-12 cells: one by the IPR model process confirmed directly with tests using PC-12 cells, and the other postulated to be a magnetic resonance-like process that is not yet sufficiently developed to give specific predictions against which to compare the available data. These results underscore the critical value of reporting not only AC field intensities, but also AC frequency, DC field intensity and relative orientation of the AC and DC fields.

Table 4:
Analysis of Variance

Source	D. F.	Mean Square	F	P
AC	3	9197.99	305.78	<.001
Error 1	10	30.08		
DC	5	490.76	40.96	<.001
AC x DC	15	573.51	47.87	<.001
H vs V	5	1137.63	94.95	<.001
H vs H&V	5	189.34	15.80	<.001
H&V vs 0.4H&V	5	505.55	42.20	<.001
0.4H&V vs V	5	348.15	29.06	<.001
H&V vs V	5	845.16	70.54	<.001
Error 2	50	11.98		

Summary

It has been demonstrated that there can be complex interrelationships between exposure conditions that must be considered in all magnetic field experiments. Fundamentally, the experimental results reported above, together with the IPR mathematical model, identify B_{ac}, B_{dc}, their relative orientations, AC frequency, and ions identified by their charge to mass ratios (q/m) as critical variables in any magnetic field exposure. This model identifies exposure parameters that may not have been consistently controlled in previous experiments. In more complex exposure scenarios, elements of these fundamental variables can be resolved from such concepts as waveform and repetition rate. Other important variables may include the electric field and induced current, the time of exposure relative to the circadian rhythm of the system, and the overall physiological state of the biological system.

Evaluation of exposure to electromagnetic conditions that can produce biological effects has been hampered until now by an incomplete knowledge of the underlying mechanism(s) that may be involved. The data reported here show clear agreement with the distinct predictions of the IPR mathematical model across variations in all key parameters. This is the first time that a theoretical model has been tested and confirmed in all fundamental relationships of those predictions. Furthermore, the degree of agreement between theory and experiment codifies the observations for the existence of a field strength "window" and now provides new basis for theoretical development.

According to the present invention, a combination of parallel oriented AC and DC magnetic fields can be used to influence a biological system (i.e., chemical mixture, organelle, cell, tissue, or organism), perhaps through an ion-molecule or ion-enzyme interaction, with the magnetic fields altering the form of that interaction for specific ions. The ions affected by the applied AC and DC magnetic fields are identified according to the mathematical equations given above. It has also been found that the hydrogen ion has a distinct role. The function of the hydrogen ion can, under certain

circumstances, suppress the magnetic field influence on the function of all other ions.

It has been demonstrated that specific relative influences of magnetic fields on biological systems can be distinctly controlled by the following variables: AC magnetic field strength, DC magnetic field strength, AC frequency and relative orientation of the AC and DC magnetic fields. Each of these variables is coupled with at least one of the other variables. The fact that the experimental data from all test cases presented herein display a response form that is consistent with the distinct, nonlinear predictions of the IPR model across different values of the critical variables demonstrates suggests that these molecular level interactions are fundamentally related to subsequent observed biological responses.

Using the IPR mathematical model described above, one can investigate and diagnose mechanisms involved in chemical and biological reactions entities such as in an organism, a group of individual cells, and even isolated organelles or chemical mixtures. Since the IPR mathematical model conforms to the predicted behavior of the application of parallel magnetic fields, one can affect the behavior of a group of cells or an organism by subjecting the entities to the influence of parallel magnetic fields. By varying three basic parameters, namely, the AC magnetic field, the DC magnetic field and the AC magnetic field frequency, one can examine on and off resonance cases.

A frequency index can be derived for each potential ion cofactor in a reaction as a function of the ion's charge-to-mass ratio and the applied B_{dc} and f_{ac} using the relationship $\underline{n} = (q \times B_{dc})/(2\pi m \times f_{ac})$. The resonance for a given ion is any combination of B_{dc} and f_{ac} that produces a frequency index ($\underline{n}$) within 10% of an integer value.

The overall response of the biological/chemical entity, for example a biological system which could be as simple as a biochemical reaction mixture or could be as complex as an organism, can be changed by exposing to parallel magnetic fields to create a resonance for a selected ion or ions. By

creating a resonance for the ion(s), the interaction of the ion(s) with its(their) biomolecular environment(s) (e.g., enzyme, transfer RNA, DNA) changes the overall response of the biological system. This response is predictable across a range of intensity values of B_{ac} (measured as peak, not rms) as defined by equation 3 above. Examples of such response include controlling the rate of tissue growth, stimulating bone growth rate, and preventing or treating osteoporosis.

Utility of Present Invention

The following illustrations of utility of the present invention assume that ions are involved in particular processes and that magnetic fields can be applied to cause those ions to be resonant as defined in the IPR mathematical model. The list of ions and oxidation states that can be considered in these examples include all of the ions noted in Table 1. As noted supra, the list in Table 1 is not exhaustive, but merely illustrative.

For diagnostic purposes, an ion is selected which is characteristic of the chemical reaction to be studied. The chemical reaction mixture is exposed to parallel magnetic fields in such a manner as to create an $n = 1$ resonance for the ion, with the peak AC flux density being 0.9 times that of the DC flux density, and the chemical reaction thereto is measured. This measurement will establish whether the selected observable can be altered by the externally applied magnetic fields. If so, then in subsequent application of those fields, the AC flux density can be changed to obtain the desired degree of alteration in system response between the maxima established in the first test and the "null" of the non-exposed case.

By measuring the chemical or biological response when an ion is stimulated at resonance, one can evaluate and alter membrane surface phenomena, such as receptor aggregation and involution and membrane components affecting surface molecules. This technique can also be used to evaluate and perhaps alter the function of membrane components, e.g., gap junction intercellular communication (gjic) as well as ion channels and pumps. Measuring gap junction intercellular communication is also useful in measuring toxic effects on developing organisms

which may lead to abnormalities. Processes occurring in the nuclei of cells can be measured and altered, including DNA conformational changes and the action of gene repressors and inducers.

By selecting at least one ion and measuring the chemical or biological response when that ion is stimulated at resonance, one can evaluate and alter processes occurring in the cytoplasm of cells. Included in these processes are activities requiring conformational states of RNA's, such as zinc fingers; enzyme activities that require ionic cofactors; protein and composite structures requiring ions for activity, such as calmodulin, cytochromes, and ribosomes.

Cellular events can be studied and altered using the techniques of the present invention. Among these are cell differentiation, such as produced by nerve growth factor action. Cell proliferation, such as segregation of chromosomes, can be studied and altered, as abnormal segregation leads to micronuclei. Ions involved in cell secretion processes can be stimulated by IPR model selected parallel magnetic fields; among the cellular components and functions associated with specific ions that can be studied include nerve synapses, immune factors and antibodies, and hormone and endocrine activities.

The process of the present invention can also be used to study the action of hormones on cells, e.g., melatonin and growth factor interaction on cell response. The effect of toxic chemicals on cells can be studied by exposing the selected ions in the cells to parallel magnetic fields and measuring the ion's response to the resonance imposed. This technique can also be used for studying the modification of cells by cellular, tissue or organism generated molecules.

Chemical and biological processes and reactions can be altered by selecting an ion which is involved in the process at issue. The process or reaction is exposed to parallel magnetic field in such a manner as to create a resonance for a selected ion or ions, such that the balance between alternative, competing chemical pathways is altered, thus leading to a different mix of products. By changing the

molecular process and reaction in this manner, one can alter the relative dynamics in interactive metabolic network of chemical reactions, such as cellular biochemical cycles.

This technology also permits control of where a reaction will occur in a sample or a body by imposing localized resonance conditions on the sample or the body. This process permits alteration of receptor-ligand interactions, including growth factors and antibodies, and changes in ion channel gating actions. At a more elemental level, imposition of resonance on a selected ion according to the present invention permits altering information at the gene level, such as induction and repression. Molecular information transfer to and status in the cytoplasm can be altered, including RNA processing and activities, as well as protein production and function.

An organism's response to chemical environmental agents can be altered by choosing an ion which interacts either with the agents or biochemical target molecules of those agents. The organism is then exposed to parallel magnetic fields so as to create a resonance for the selected ion. The strength and relationship of the magnetic fields are altered so as to diminish the organisms response to opioids or to free radicals. Additionally, the magnetic fields can be chosen so as to reinforce activity in selected locations in the brain to enhance memory retention.

By studying and altering gap junction intercellular communication using imposition of selected ion resonance on a cell assembly, tissue, organ, or organism, the toxicity of chemicals can be altered. Enhancing the activity of a cytotoxic chemical, particularly at a selected site in an organisms, can inhibit or treat cancerous cells. The chemical treatment which destroys rapidly growing cancer cells is enhanced by application of the invention to alter the functions of gap junctions, ion channels or receptor signaling systems, and by damaging cell division processes. Enhanced damage to cancer cells is also useful in inhibiting metastasis of cancer cells. By augmenting chemotherapy in a controlled way, the required amount of chemotherapeutic chemical(s) can be reduced,

thereby subjecting normal tissue to lower levels of these toxic substances, giving an improved chance of survival for the organism. Thus, the process of the present invention can be used in conjunction with conventional chemotherapy in treating cancer.

Imposition of resonance on a selected ion can be used to modulate hormonal action on cell processes, the ion being selected based upon the cellular ions involved or upon the ion involved in the hormonal action. Stimulation of ion resonance can also be used to alter tissue and organ system functions, such as learning (acquisition of information), memory (retention of information), and immune response to challenges.

The action of growth factors can be altered by imposing resonance on at least one selected ion in the growth factor stimulated system. An example of this is altering the role of nerve growth factor in maturation and repair of cells. Imposition of resonance on a selected ion in a cell or tissue can be used to alter cell surface events in immunological response, such as challenge from an infectious agent, or stimulation by autoimmune processes.

Creation of ionic resonance conditions in small selected volumes within a body can be used to activate biologically active compounds directly at a particular site in the body. This is of particular importance in delivery of chemotherapeutic drugs, which are often toxic to healthy cells as well as to tumor cells. The biologically active compound is attached to a carrier molecule or component which has an ionic cofactor necessary for the entrapment of the compound. Imposition of an appropriate magnetic field at the target site can change the ionic interactions, causing the conjugate molecule to release the active compound from the carrier molecule or component thereof. Additionally, a detectable label may be added to the ionic cofactor to determine when the conjugate has reached the target site. This determines when appropriate fields can be applied.

Examples of carrier components include molecules and membrane vesicles to transport chemicals. Alternatively, the chemical could be in an inactive state and rendered active at

the site by imposition of the resonance on a selected ion complexed with the chemical. A chemotherapeutic drug is contained in liposome-like vesicles which contain ion-controlled pores that open under resonance conditions. Drugs can be engineered to be inactive under normal conditions, but active when they are within a volume in which the resonance condition exists.

As noted above, to use IPR methodology, an ion is selected which is characteristic of the chemical reaction to be studied or altered. The ion is exposed to parallel magnetic fields in such a manner as to create a resonance for the ion, and the degree of change in the chemical reaction thereto desired is controlled by adjusting the applied AC magnetic flux density. For diagnostic purposes, a variety of chemical steps in the movement of information from the cell's exterior to the nucleus, via signal transduction processes, are susceptible to alteration by magnetic field which affect ionic components in these processes. This alteration of the ionic components is thus used to reveal the parts of the processes that are involved as well as their cellular locations. The ion-related processes that bring instructions from the genes to the cytoplasm and are involved in the assembly and function of molecular components to perform specific cellular functions can conceivably also be examined and altered or controlled using IPR methodology.

Imposition of resonance on an ion and subsequent controlled alteration in response using IPR methodology can be used to selectively change chemical/biochemical reactions and thus the interaction of a biological system with its environment. Alteration in specific ion-related biochemical steps in complex, interactive metabolic pathways can cause changes in final reaction products and thus in biological processes, including integrated functions of an organisms.

More specific examples of applications of IPR methodology are given below. However, these examples are given solely for purposes of illustration, and are not meant to be exclusive or exhaustive.

Cell Surface Phenomena

To examine the effects of medium components on cell surface phenomena, primed PC-12 cells were stimulated with nerve growth factor to produce neurites and were exposed for 23 hours to IPR resonance conditions: 45 HZ AC between 132 and 344 mG (rms), with a DC field of 366 mG parallel and <2mG perpendicular to the AC field. Cells prepared in a standard medium, RPMI 1640, exhibited the U-shaped dose response described above. However, cells prepared in Iscoves' medium exhibited very nearly no response, similar to off-resonance test results. The major difference in medium components between the two media is HEPES, a buffer known to interfere with some cell surface properties (see Figure 9).

Other cell surface phenomena, including receptor aggregation and involution, are susceptible to perturbation and thus examination and control, by IPR methodology as described above.

Function of Membrane Components

To examine the function of membrane components, the influence of IPR resonance conditions on the capability of cells to perform gap junction intercellular communication were examined. In this case the resonance condition for predicted maximal response was 45 HZ at 238 mG(rms), with a DC field of 366 mG parallel and <2mG perpendicular to the AC field. A rat-liver cell line, Clone 9, was treated with chloral hydrate to partially inhibit intercellular communication as demonstrated by the transfer of the fluorescent dye, Lucifer yellow, using the standard scrape/load assay. After exposure for 30 minutes these cells produced more inhibition of intercellular communication as shown in Figure 10. Exposure to the various AC flux densities between 152 and 318 mG(rms) produced inhibition which closely followed the U-shaped dose response demonstrated by neurite inhibition in nerve growth factor stimulated PC-12 cells under similar exposure conditions. This result now suggests a means to investigate further the processes controlling intercellular communication. In the same manner, the IPR methodology can be applied to investigate and regulate the functioning of ion channels, which often have

transition metal cofactors in gating regions, as well as ion pump processes which actively segregate ions between different cell compartments and the exterior of the cell.

Other Processes

Other critical processes in the cell that are susceptible to diagnosis and control by IPR methodology are those which involve an ion that can be subjected to resonance. DNA molecules in the nucleus are complexed with molecules that act as structural and control elements for reading the genetic code. Ions, including magnesium, manganese and hydrogen, have central roles in providing forces to maintain and alter DNA conformation, and for information processing of genes. Likewise, biochemical processes in the cytoplasm of cells can be investigated using IPR methodology. Some transfer RNAs use zinc complexes to confer selectivity in function. Hydrogen is important for conformational specificity, and may be examined for the relative stability it confers on the RNA. Furthermore, some enzyme activities require ionic cofactors and hydrogen, as do some protein and composite structures, e.g., calmodulin, cytochromes, and ribosomes. These sites are ideal for the magnetic field-based intervention via IPR methodology to distinguish, differentiate, and regulate by selected amounts the roles of ions in the natural processes of these molecules.

Cell differentiation is a crucial process in development and tissue repair, which causes the growth and integration of different, specialized functional parts of an organism. Some differentiation processes are stimulated by growth factors which act through specific receptors in or on the cell. The process of magnetic field control of nerve growth factor stimulated neurite growth via IPR model specified field parameters has been studied in a cell culture system of PC-12 cells as describe above. The results of these experiments, in which PC-12 cells stimulated with nerve growth factor are exposed to a prescribed set of parallel AC and DC magnetic fields for 23 hours, demonstrated that hydrogen ions and at least one of magnesium, manganese and vanadium ions, are involved with molecules critical to the differentiation process.

Cell differentiation processes can be modulated by hormones. The action of the hormone, melatonin, was examined on the nerve growth factor stimulation of neurite outgrowth. PC-12 cells stimulated with nerve growth factors were exposed to magnetic fields for 23 hours at an IPR-designated maximal effect {45 Hz, AC at 238 mG(rms), DC field at 366 mG parallel and <2 mG perpendicular to the AC field}. Different concentrations of melatonin modulated the effects of this resonance condition. This demonstrated the influence of melatonin on the growth factor stimulation process which occurred at physiological concentrations of melatonin. Additionally, the action of physiological concentrations of melatonin were shown to ameliorate the reduction of gap junction intercellular communication in Clone 9 cells from the rat liver that had been treated with chloral hydrate and exposed for 30 minutes to an IPR-designated maximum effect {45 Hz, AC at 238 mG(rms), DC field at 366 mG parallel and <2 mG perpendicular to the AC field}. Changes in intercellular communication are causally linked to differentiation processes. The results described above showing IPR model specified combinations of parallel magnetic fields controlling cell changes induced by melatonin have never been reported before, and demonstrate that melatonin has previously unknown actions on cellular level processes that can be controlled in the distinct manner described by the IPR model.

Since gap junction intercellular communication is a fundamental process by which cells coordinate actions during embryogenesis, IPR methodology can be used to understand and regulate the processes by which chemicals cause abnormalities during development. Further, since intercellular communication is important to maintain homeostasis and prevent abnormal growth in the mature organisms, IPR methodologies can be incorporated into studies investigating and controlling the causes and processes involved with carcinogenesis and other abnormal growth processes.

The cells in many tissues in the body undergo frequent division to replenish natural losses, as in the intestinal lining and in the immune system, and when tissues

must undergo repair or replace cells in tissues, such as when glial cell proliferation occurs in response to neuron death caused by selective toxic chemical damage. One essential operation of cell proliferation is the segregation of chromosomes into two compartments. This segregation is accomplished by a molecular fiber system that draws duplicate chromosomes to opposite positions before the cell is partitioned in two. The processes producing this movement of chromosomes to opposite poles require the involvement of ions. IPR methodology can be used to establish which ions are involved, which are particularly labile to perturbation by resonant magnetic fields and to precisely regulate those processes via control of the applied parallel AC magnetic field flux density. Data already in the literature on micronuclei formation in human peripheral lymphocytes indicate that this process as well is amenable to investigation and control by IPR methodology.

Molecular processes and reactions can be differentially influenced by resonant magnetic fields, thereby altering the relative dynamics in linked chemical and biochemical reactions. IPR methodology can be applied to localized regions within a body, for selected maximal and minimal perturbation of ongoing reactions and processes. For example, a DC field of 366 mG parallel and <2 mG perpendicular to a 45-Hz AC field of 238 mG (rms) would cause a maximal effect for a set of tuned ions. However, by virtue of gradients in AC field flux densities easily created by an exposure apparatus, AC fields of 492 or 919 mG(rms) could be generated that would produce no effect. The feasibility of this approach has been demonstrated by the stacking of dishes containing PC-12 cells stimulated with NGF in an AC intensity gradient under constant DC field conditions where cells in AC intensities less than optimum predicted by IPR conditions produce less neurite outgrowth response. Alternatively, under constant AC flux densities, gradients in the DC field can be produced that place parts of the exposed system in or out of resonance conditions, thus causing an effect or not causing an effect.

Receptor-ligand interactions, which occur with growth factor stimulation for receptors, can be altered by IPR methodologies. This application has been demonstrated as described above with primed PC-12 cells stimulated with nerve growth factor to produce neurites. Cells exposed for 23 hours to IPR resonance conditions, in this case 45 Hz AC between 132 and 344 mG(rms) with a DC field of 366 mG parallel and <2 mG perpendicular to the AC field, gave the generally U-shaped dose response predicted by the IPR model. This response is selective because the stimulation of neurite outgrowth by basic fibroblast growth factor was observed in separate tests to not be affected by approximately resonant IPR exposure conditions.

Natural and toxic biological processes can be altered by IPR-defined resonant magnetic fields. The toxicity of chemicals can be enhanced, as demonstrated by the further reduction in gap junction intercellular communication in chloral hydrate-treated Clone 9 rat liver cells as described above. Reduction of intercellular communication in chloral hydrated cells is consistent with reduced growth control and thus an increased potential for cancer promotion.

The action of growth factors in maturation and repair can be modulated by IPR resonant fields. For example, primed PC-12 cells stimulated with nerve growth factor to produce neurites, and exposed for 23 hours to IPR resonance conditions as described above, produced varying degrees of inhibition of the stimulation depending upon the AC flux density applied, exactly as predicted by the IPR model. A subclone of PC-12 cells, PC-12D, which has greater concentrations of the TRK in the membrane, showed stimulation of neurite outgrowth by near IPR resonant exposure conditions without the presence of nerve growth factor. Thus IPR methodologies can be expected to be used in certain biological circumstances to enhance nerve regeneration after injury or trauma.

It is possible that the IPR methodology, which identifies combinations of AC and DC magnetic fields that can reduce growth control processes, can be used to augment chemotherapy by enhancing the number of rapidly growing cancer cells by altering intercellular communication thereby making

them more susceptible to a chemotherapuetic agent. IPR methodology thus can be used either directly or in combination with chemotherapeutic agents to damage cell division processes in a precisely controllable manner, including regulation of enhanced micronuclei formation in human peripheral lymphocytes.

As noted above, hormone action on cell processes can be modulated using IPR. The hormone melatonin has been shown to enhance intercellular communication in C3H 10T1/2 cells, derived from a mouse embryo (Ubeda et al., 1995a). Morphological transformation in this cell line, which includes altered intercellular communication, is used to identify potential cancer promoting chemicals. High intensity magnetic fields removed the enhancement in intercelluar communication caused by the melatonin (Ubeda et al., 1995b). Pilot data show the effect also occurs near an optimum in IPR-defined conditions (45 Hz AC at 660 mG(rms), and DC field at 366 mG parallel and <2 mG perpendicular to the AC field). This is also supported by the known ability of physiological concentrations of melatonin to counter the reduction in IC chloral hydrate-treated Clone 9 cells caused by optimal IPR conditions (45 Hz AC at 238 mG(rms), DC field at 366 mG parallel and <2mG perpendicular to the AC field).

Magnetic fields penetrate biological systems without any appreciable attenuation. Cell and tissue functions that depend on ion cofactors can be altered by IPR-defined resonant conditions for pertinent ions. The acquisition of information (learning) and the retention for information (memory) are known to be influenced by calcium and magnesium ions, among others. Pilot data are consistent with an IPR-defined resonant control of these ions during rodent performances on tasks designed to evaluate learning and memory. There are numerous potential applications of IPR methodologies in the neurosciences research and clinical areas. The influence of IPR-defined exposure conditions on cell membrane processes indicate the immune response in numerous categories might be controlled, either positively or negatively, when magnetic fields are tuned for specific ions and the AC flux density adjusted to produce the

desired inhibition/stimulation of the selected process. Thus, immune defence against various disease agents or aberrant cells may be subject to alteration by IPR methodologies. Further, drugs and/or delivery systems can be engineered either to preferentially activate or deposit drugs at desired sites within the body using selected ion-related resonance conditions defined by the spatial gradient features of IPR methodology.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation.

Abrams, MJ, Murrer, BA (1993): Metal compounds in therapy and diagnosis. Science 261: 725-730.

Adair, RK (1991) Constraints on biological effects of weak extremely-low- frequency electromagnetic fields. Physical Review A 43: 1039-1048.

Adair, RK (1992) Criticism of Lednev's mechanism for the influence of weak magnetic fields on biological systems. Bioelectromagnetics 13:231-235.

Adey, WR (1992): Collective properties of cell membranes. In Norden B, Ramel C (eds): "Interaction Mechanisms of Low-Level Electromagnetic Fields in Living Systems." Oxford: Oxford University Press, pp 47-77.

Bialek, W, Bruno, WJ, Joseph J, Onuchic, JN (1989) Quantum and classical dynamics in biochemical reactions. Photosynthesis Research 22: 15-27.

Bianco, B, Chiabrera, A (1992) From the Langevin-Lorentz to the Zeeman model of electromagnetic effects on ligand-receptor binding. Bioelectrochemistry and Bioenergetics 28:355-365.

Blackman, CF, Blanchard, JP, Benane, SG, House, DE (1995) The ion parametric resonance model predicts magnetic field parameters that affect nerve cells. FASEB J 9:547-551.

Blackman, CF, Blanchard, JP, Benane, SG, House, DH (1994) Empirical Test of an Ion Parametric Resonance Model for Magnetic Field Interactions with PC-12 Cells. Bioelectromagnetics 15:239-260.

Blackman, CF (1992) Calcium release from neural tissue: experimental results and possible mechanisms. In Norden B, Ramel C (eds): "Interaction Mechanisms of Low-Level

Electromagnetic Fields in Living Systems." Oxford: Oxford University Press, pp. 107-129.

Blackman, CF, Benane, SG, Rabinowitz, JR, House, DE, Joines, WT (1985) A role for the magnetic field in the radiation-induced efflux of calcium ions from brain tissue, in vitro. Bioelectromagnetics 6: 327-337.

Blackman, CF, Benane, SG, Elliott, DJ, House, DE, Pollock, MM (1988) Influence of electromagnetic fields on the efflux of calcium ions from brain tissue in vitro: A three-model analysis consistent with the frequency response up to 510 Hz. Bioelectromagnetics 9(3): 215-227.

Blackman, CF, Benane, SG, House, DE, Elliott, DJ (1990) Importance of alignment between local DC magnetic field and an oscillating magnetic field in responses of brain tissue in vitro and in vivo. Bioelectromagnetics 11(2):159-167.

Blackman, CF, Benane, SG, House, DE (1993) Evidence for direct effect of magnetic fields on neurite outgrowth. FASEB J, 7:801-806.

Blackman, CF, Benane, SG, Kinney, LS, Joines, WT, and House, DE (1982) Effects of ELF fields on calcium-ion efflux from brain tissue in vitro. Radiation Research 92:510-520.

Blackman, CF, Kinney, LS, House, DE, and Joines, WT (1989) Multiple power-density windows and their possible origin. Bioelectomagnetics (10(2):115-128.

Blackman, CF, Benane, SG (1995b) Frequency-dependent interference by magnetic fields of nerve-growth-factor-induced neurite outgrowth in PC-12 cells. Bioelectromagnetics, accepted for publication.

Blanchard, JP, Blackman, CF (1994) Clarification and Application of an Ion Parametric Resonance Model for Magnetic Field Interactions with Biological Systems. Bioelectromagnetics 15:217-238.

Blanchard, JP, House, DH, Blackman, CF (1994a) Evaluation of Whole Animal Data using the Ion Parametric Resonance Model. Bioelectromagnetics, accepted for publication.

Blanchard, JP, Blackman, CF (1994b) A model of magnetic field effects on biological systems with confirming data from a cell culture preparation. In Frey, AH (ed): "On the nature of electromagnetic field interactions with biological systems." R.G. Landes: USA, 1994, pp. 43-58.

Blanchard, JP, Blackman, CF, Benane, SG, House, DE (1994c) Resonance bandwidth under IPR model exposure conditions. The 1994 Annual Review of Research on Biological Effects of Electric and Magnetic Fields from the Generation, Delivery and Use of Electricity, November 6-10, 1994, Albuquerque, NM.

Chiabrera, A, Bianco, B (1991) Quantum dynamics of ions in molecular crevices under electromagnetic exposure. In Brighton, CT, Pollack, SR (eds): "Electromagnetics in Biology and Medicine." San Francisco: San Francisco Press, pp 21-26.

Chiabrera, A, Bianco, B, Kaufman, JJ, Pilla, AA (1991) Quantum analysis of ion binding kinetics in electromagnetic bioeffects. In Brighton, CT, Pollack, SR (eds): "Electromagnetics in Biology and Medicine." San Francisco: San Francisco Press, pp 27-31.

Chiabrera, A, Bianco, B, Moggia, E (1993) Effect of lifetimes on ligand binding modelled by the density operation. Bioelectrochemistry and Bioenergetics 30:35-42.

Durney, CH, Rushforth, CK, Anderson, AA (1988) Resonant DC-AC magnetic fields: calculated response. Bioelectromagnetics 9(4):315-336.

Edmonds, DT (1993) Larmor precession as a mechanism for the detection of static and alternating magnetic fields. Bioelectrochemistry and Bioenergetics 30: 3-12.

EPRI (1990) The Cyclotron Resonance Hypothesis: An EMF Health Effects Resource Paper, Electric Power Research Institute, Palo Alto, CA, EN.3014.3.90.

Frauenfelder, H, Parak, F, Young, RD (1988) Conformational substates in proteins. Ann Rev Biophys Biophys Chem 17: 451-479.

Greene, LA (1977) A quantitative bioassay for nerve growth factor (NGF) activity employing a clonal pheochromocytoma cell line. Brain Research 133:350-353.

Greene, LA and Tischler, AS (1976) Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc. Natl. Acad. Sci. (USA) 73:2424-2428.

Halle, B (1988) On the cyclotron resonance mechanism for magnetic field effects on transmembrane ion conductivity. Bioelectromagnetics 9: 381-385.

Karlin, KD (1993) Metalloenzymes, structural motifs, and inorganic models. Science 261: 701-708.

Karplus, M, Brunger, AT, Elber, R, Kuriyan, J (1987) Molecular Dynamics: Applications to Proteins. Cold Spring Harbor Symposia on Quantitative Biology, Volume LII, Cold Spring Harbor Laboratory, 381-390.

Karplus, M, Petsko, GA (1990) Molecular dynamics simulations in biology. Nature 347: 631-639.

Landreth, GE, Smith, DS, McCabe, C, Gittinger, C (1990) Characterization of a nerve growth factor-stimulated protein kinase in PC12 cells which phosphorylates microtubule-associated protein 2 and pp250. J. Neurochem. 55:514-523.

Lednev, VV (1991) Possible mechanism for the influence of weak magnetic fields on biological systems. Bioelectromagnetics 12:71-75.

Lerchl, A, Reiter, RJ, Howes, KA, Nonaka, KO, Stokkan, K-A (1991) Evidence that extremely low frequency Ca2+ - cyclotron resonance depresses pineal melatonin synthesis in view. Neuroscience Letters, 124:213-215.

Levi, A, Biocca, S, Cattaneo, A, Calissano, P (1988) The mode of action of nerve growth factor in PC12 cells. Molecular Neurobiology 2:201-226.

Liboff, AR (1985) Cyclotron resonance in membrane transport. In Chiabrera, A, Nicolini, C, Schwan, HP (eds): "Interactions between Electromagnetic Fields and Cells." NATO ASI Series A97. New York: Plenum, pp 281-296.

Liboff, AR, Smith, SD, McLeod, BR (1987) Experimental evidence for ion cyclotron resonance mediation of membrane transport. In Blank M, Findl E (eds), "Mechanistic approaches to interactions of electric and electromagnetic fields with living systems." New York: Plenum, pp 109-132.

Liboff, AR, Parkinson, WC (1991) Search for ion-cyclotron resonance in an Na^+-transport system. Bioelectromagnetics 12:77-84.

Liboff, AR (1992) The 'cyclotron resonance' hypothesis: experimental evidence and theoretical constraints. In Norden, B, Ramel, C (eds): "Interaction Mechanisms of Low-Level Electromagnetic Fields and Living Systems". Oxford: Oxford University Press, pp 130-147.

Liburdy, RP, Sloma, TR, Sokolic, R, Yaswen, P (1993) ELF magnetic fields, breast cancer, and melatonin: 60 Hz fields block melatonin's oncostatic action on ER+ breast cancer cell proliferation. J. Pineal Res., 14:89-97.

Lippard, SJ (1993) Bioinorganic chemistry: a maturing frontier. Science 261: 699-700.

McLeod, BR, Liboff, AR (1987) Cyclotron resonance in cell membranes; the theory of the mechanism. In Blank M, Findl E (eds), "Mechanistic approaches to interactions of electric and electromagnetic fields with living systems." New York: Plenum, pp 97-108.

O'Halloran TV (1993) Transition metals in control of gene expression. Science 261: 715-725.

Podgoretskii, MI, Khrustalev, OA (1964) Interference effects in quantum transitions. Soviet Physics Uspekhi 6(5): 682-700.

Pyle, AM (1993) Ribozymes: a distinct class of metalloenzymes. Science 261: 709-714.

Regan, L (1993) The design of metal-binding sites in proteins. In Engelman, DM, Cantor, CR, Pollard, TD (eds): "Annual Review of Biophysics and Biomolecular Structure." Palo Alto: Annual Reviews, Inc, pp 257-281.

Ross, SM (1990) Combined dc and ELF Magnetic Fields Can Alter Cell Proliferation. Bioelectromagnetics 11: 27-36.

Rukenstein, A, Greene, LA (1983) The quantitative bioassay of nerve growth factor: use of frozen 'primed' PC12 pheochromocytoma cells. Brain Research 263:177-180.

Sandweiss, J (1990) On the cyclotron resonance model of ion transport. Bioelectromagnetics 11: 203-205.

Sandyk, R (1993) Weak magnetic fields antagonize the effects of melatonin on blood glucose levels in Parkinson's disease. Intern. J. Neuroscience, 68:85-91.

Sano, M (1992) Chromatographic resolution and characterization of a nerve growth factor-dependent kinase that phosphorylates microtubule-associated proteins 1 and 2 in PC12 cells. J. Neurochem. 59: 1263-1272.

Smith, SD, McLeod, BR, Liboff, AR, Cooksey, K (1987) Calcium cyclotron resonance and diatom mobility. Bioelectromagnetics 8(3): 215-227.

Thomas, JR, Schrot, J, Liboff, AR (1986) Low-intensity magnetic fields alter operant behavior in rats. Bioelectromagnetics 7: 349-357.

Trillo, A, Ubeda, A, Blanchard, JP, House, DE, Blackman, CF (1994) Effects of AC/DC magnetic fields under resonant conditions for the hydrogen ion on the NGF-induced neurite outgrowth in PC-12 cells. Abstract accepted for presentation at the 16th Annual Meeting of the Bioelectromagnetics Society, Copenhagen, Denmark, 12-17 June 1994.

Ubeda, A, Trillo, MA, House, DE, Blackman, CF (1995) Melatonin enhances junctional transfer in normal C3H/10T1/2 cells. Cancer Letters, in press.

Ubeda, A, Trillo, MA, House, DE, Blackman, CF (1995) A 50-Hz magnetic field blocks melatonin-induced enhancement of junctional transfer in normal C3H/10T1/2 cells. Submitted to Carcinogenesis.

Yariv, A (1982) "An Introduction to Theory and Applications of Quantum Mechanics", John Wiley and Sons, Inc., New York, pp. 232-233.

WHAT IS CLAIMED IS:

1. A method for altering the action of a system selected from the group consisting of chemical systems and biological systems comprising exposing the system to a predetermined combination of AC and DC magnetic fields.

2. The method according to claim 1 wherein said system is selected from the group consisting of chemical compounds, enzymes, cells, organs, organisms, and mixtures thereof.

3. The method according to claim 1 wherein the magnetic field includes parallel AC and DC magnetic fields.

4. The method according to claim 3 wherein the magnetic field comprises a DC magnetic field and an AC magnetic field and the flux density of the DC magnetic field is minimized perpendicular to the AC magnetic field while the DC magnetic field flux density parallel to the AC magnetic field is adjusted to the value required for ion resonance.

5. The method according to claim 4 wherein the AC magnetic field flux density is adjusted to produce a controlled degree of response in said system relative to what would be observed in said system without exposure to the magnetic field.

6. The method according to claim 5 wherein the degree of change in observed response, relative to an unexposed biological system that is otherwise identical to the exposed system, is given by a weighted sum of Bessel functions of the first kind, the order to said Bessel functions being selected according to an integer value of the frequency index of an ion in the biological system, where the frequency index of the ion is defined as the charge to mass ratio of the ion times the flux density of the DC magnetic field divided by the angular frequency of the AC magnetic field.

7. The method according to claim 5 wherein the Bessel function argument is of the form

$x=(2\circ\underline{n}\circ B_{ac}(pk)/B_{dc})$, where

$\underline{n}$= frequency index

$B_{ac}(pk)$ = the peak value of the AC magnetic field

flux density, and

B_{dc} = the value of the DC magnetic field flux density.

8. A method for examining and altering reactions in a system exposed to AC and DC magnetic fields comprising:

(a) selecting at least one ion to be stimulated within said system;

(b) exposing said system to parallel magnetic fields to create a resonance for said at least one ion, wherein the AC frequency and the DC field strength are adjusted to create said resonance;

(c) measuring the response of said system to the resonance of said at least one ion;

(d) measuring the response of said system when no magnetic fields are applied; and

(e) comparing the responses of said system in steps (c) and (d).

9. A method for examining and altering reactions in a system selected from the group consisting of chemical systems and biological systems treated with an agent selected from the group consisting of chemical agents and physical agents comprising:

(a) measuring the changes of said system over a predetermined period of time without any external perturbation of said system;

(b) selecting at least one ion to be stimulated in said system;

(c) selecting a combination of parallel AC and DC magnetic fields to create a resonance for said at least one ion;

(d) applying said agent to said system;

(e) measuring the response of said system to said agent; and

(f) exposing the system of step (d) to the parallel AC and DC magnetic fields of step (c);

(g) measuring the response of the system; and

(h) comparing the responses measured in steps (a),

(e), and (g).

10. The method according to claim 9 wherein said agent is a natural or a synthetic hormone.

11. The method according to claim 9 wherein said agent is melatonin.

12. The method according to claim 9 wherein said agent is a growth factor.

13. The method according to claim 12 wherein said growth factor is nerve growth factor.

14. The method according to claim 9 wherein said agent is a cytotoxic compound.

15. The method according to claim 2 wherein the magnetic field includes perpendicular AC and DC magnetic fields.

16. The method according to claim 15 wherein the degree of change in observed response, relative to an unexposed system that is otherwise identical to the exposed system, is the opposite direction of that observed under parallel field exposures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/12343

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 13/00
US CL :435/173.1, 173.2, 173.8
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/173.1, 173.2, 173.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CA, BIOSIS, EMBASE, MEDLIN
Search terms: magnet? field#, melatonin, biological, parallel, blackman

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US, A, 5,045,050 (LIBOFF ET AL.) 03 September 1991, see example 1 and column 20, lines 36-43.	8-10, 12-13 --- 11
X	US, A, 5,087,336 (LIBOFF ET AL.) 11 February 1992, see Figure 3, equation 2, column 7, lines 14-18, and columns 11-12.	1-8, 15-16
Y	J. PINEAL RES., Volume 14, issued 26 May 1993, R.P. Liburdy et al. "ELF Magnetic Fields, Breast Cancer, and Melatonin: 60 Hz Fields Block Melatonin's Oncostatic Action of ER+ Breast Cancer Cell Proliferation", pages 89-97, see entire document.	11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search
14 DECEMBER 1995

Date of mailing of the international search report
02 JAN 1996

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
JON P. WEBER, PH.D.
Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/12343

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NEUROSCI. RES., Volume 124, Number 2, issued 1991, A. Lerchl et al., "Evidence That Extremely Low Frequency Ca2+-Cyclotron Resonance Depresses Pineal Melatonin Synthesis In Vitro", pages 213-215, see entire document.	11
Y	INTERN. J. NERUOSCI., Volume 68, Number 1-2, issued 1993, R. Sandyk, "Weak Magnetic Fields Antagonize the Effects of Melatonin on Blood Glucose Levels in Parkinson's Disease", pages 85-91, see entire document.	11